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Master's thesis

Asymmetric Synthesis of (*R*)-(-)-*O*-desmethylangolensin using (*R*,*R*)-(-)-Pseudoephedrine as Chiral Auxiliary

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Abstract

O-Desmethylangolensin (*O*-DMA) is a product of anaerobic intestinal bacterial metabolism of the isoflavone daidzein, which is found mainly in soy foods. Because of its structural similarity to natural estrogen, it was found to have a strong binding affinity to human estrogen receptor β, thus explaining its anticarcinogenic activity among other biological actions. Because of its biological importance, the main aim of this study is to propose a synthetic route using (*R*,*R*)-(-)-Pseudoephedrine as chiral auxiliary to synthesize enantiopure (*R*)-(-)-*O*-DMA. Pseudoephedrine has been previously found to be an excellent chiral auxiliary; its amide is stable, easily prepared and its enolate is highly reactive and can undergo many useful transformations including alkylation reactions.

4-Hydroxyphenylacetic acid was used as starting material. Diastereoselective α-methylation of the pseudoephedrine amide was done using LDA in THF with subsequent addition of methyl iodide. The method was found successful; however, the yield (55%) and % ee (4.5%) were low, thus further adjustments are needed.

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Acknowledgments

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List of abbreviations

acac	**Acetylacetone**
Ar	**Aryl**
Bcl-2	**B-cell lymphoma 2**
Bax	**Bcl-2 associated X protein**
Bn	**Benzyl**
BnBr	**Benzyl bromide**
BINAP	**(2,2'-bis(diphenylphosphino)-1,1'-binaphthyl)**
CBDTS	**1,2-bis(diphenylphosphinomethyl)cyclobutane**
BDDPTS	**2,4-bis(diphenylphosphino)pentane**
R-SMS-Phos	**1,2-bis[(*o*-RO-phenyl)(phenyl)phosphino]ethane**
$BF_3.Et_2O$	**Boron trifluoride diethyl etherate**
b/l	**Branched/linear ratio**
***n*-BuLi**	***n*-Butyllithium**
$BMI.BF_4$	**1-n-butyl-3-methylimidazolium tetrafluoroborate**
CDK	**Cyclin dependent kinase**
Cy	**Cyclohexyl**
cod	**1,5-Cyclooctadiene**
cPent	**Cyclopentyl**
***O*-DMA**	***O*-desmethylangolensin**
DCM	**Dichloromethane**
EI-MS	**Electron impact mass spectrometry**
Equiv./eq.	**Equivalent(s)**
Et_2O	**Diethyl ether**
***n*-DIPA**	***n*-Diisopropylamine**
***i*-Pr_2NEt**	**Diisopropylethylamine**
% ee	**% enantiomeric excess**
EtOH	**Ethanol**
EtOAc	**Ethyl acetate**
FSH	**Follicle stimulating hormone**
GC-MS	**Gas chromatography-mass spectrometry**
GIT	**Gastrointestinal tract**
GC	**Glassy carbon**
G_1 phase	**Growth 1/Gap 1 phase**
G_2 phase	**Gap 2/Pre-mitotic phase**
LiHMDS	Lithium bis(trimethylsilyl)amide
G_1/S	**Transition between G_1 and S phases**
G_2/M	**Transition between G_2 and M phases**
HDL	**High density lipoprotein**
hER	**Human Estrogenic Receptor**

IC_{50}	**50% Inhibitory concentration**
LDA	**Lithium diisopropylamide**
LDL	**Low density lipoprotein**
M phase	**Mitotic phase**
MeOH	**Methanol**
MCF-7	**Michigan Cancer Foundation-7**
NMR	**Nuclear magnetic resonance**
OVX	**Ovariectomized**
PEG	**Polyethylene glycol**
rt	**Room temperature**
SPO	**Secondary phosphine oxide**
SpinPHOX	**spiro[4,4]-1,6-nonadienebased phosphine–oxazoline**
S phase	**Synthesis phase**
THF	**Tetrahydrofuran**
TetraMe-BITIOP	**2,2',5,5'-tetramethyl-4,4'-bis- (diphenylphoshino)-3,3'-bithiophene**
TLC	**Thin layer chromatography**
TsDPEN	***N*-(*p*-toluenesulfonyl)-1,2-diphenylethylenediamine**
TC	**Total cholesterol**
TM	**Transition metal**
Et_3N	**Triethylamine**
TG	**Triglycerides**

1 Introduction

1.1 *O*-desmethylangolensin

1.1.1 Sources

Isoflavones and lignans are plant-derived secondary metabolites, which are frequently consumed in the diet and are believed to benefit human health. Whereas lignans are found in fiber-rich foods such as: whole grains and seeds (flaxseed, linseed and sesame seeds), the most common sources of dietary isoflavones are soybeans and their products including soy flour, soy milk, miso, tofu and tempeh. Isoflavones and lignans as well as their metabolites belong to a class of compounds referred to as phytoestrogens, which from the name implies their ability to bind to estrogen receptors considering their structural similarity to natural 17*β*-estradiol **4**.[1,2] *O*-Desmethylangolensin **3** (*O*-DMA) and equol **2**, first identified in human urine in the 1980s, were later recognized to be the intestinal bacterial metabolic products of the soy isoflavone daidzein **1**.[3] Figure 1 shows the structures of 17*β*-estradiol **1**, the isoflavone daidzein **2** and its major metabolites, equol **3** and *O*-DMA **4**.

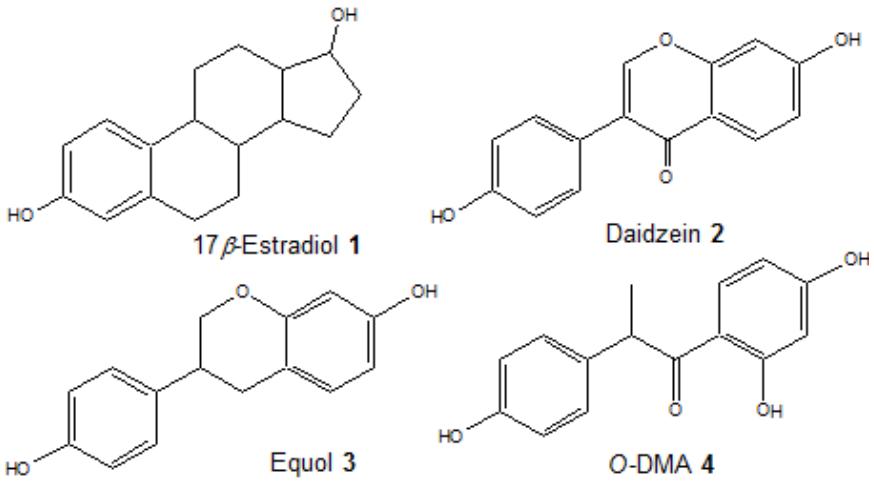


Figure 1: Structures of 17β-estradiol (E_2), daidzein, equol and O-DMA.[3]

1.1.2 Production and metabolism

Modification of the soy isoflavones by the intestinal bacteria produces metabolites that might have a more potent, or even different, biological actions than their compounds. Thus, the metabolic fate of these isoflavones directly affects their biological activity.[4,5] As mentioned above, the precursor of *O*-DMA and equol is daidzein **2**, found in fermented soy foods, or the glycoside form daidzin, found in unfermented soy foods. After ingestion, daidzin can be hydrolyzed in the small intestine by *β*-glycosidase to form the aglycone daidzein **2**, which is further metabolized by anaerobic bacteria in the large intestine into dihydrodaidzein.[3,7] Other intestinal bacteria then reduce this compound to equol **3** or cause ring cleavage to form *O*-DMA **4** (Figure 2). *O*-DMA can then be absorbed and conjugated to the glucuronide/sulfate in the liver/intestinal mucosa to make it water soluble, which can then be released from the liver into the circulation and finally excreted by the kidneys into the urine. Also, a part can be released by the liver into the bile, where it undergoes enterohepatic circulation (Figure 3).[3]

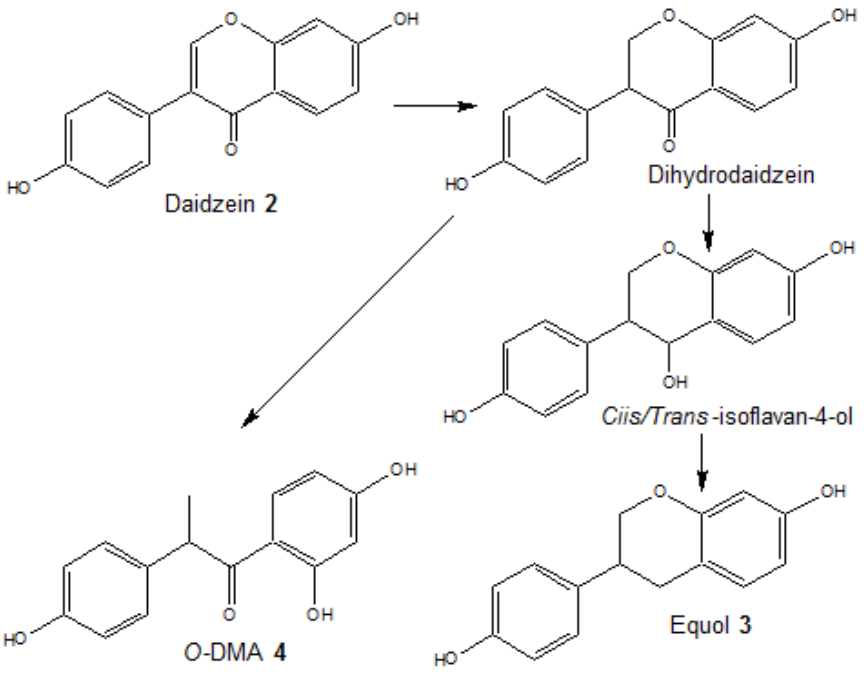


Figure 2: Metabolism of daidzein to equol and O-DMA.[4]

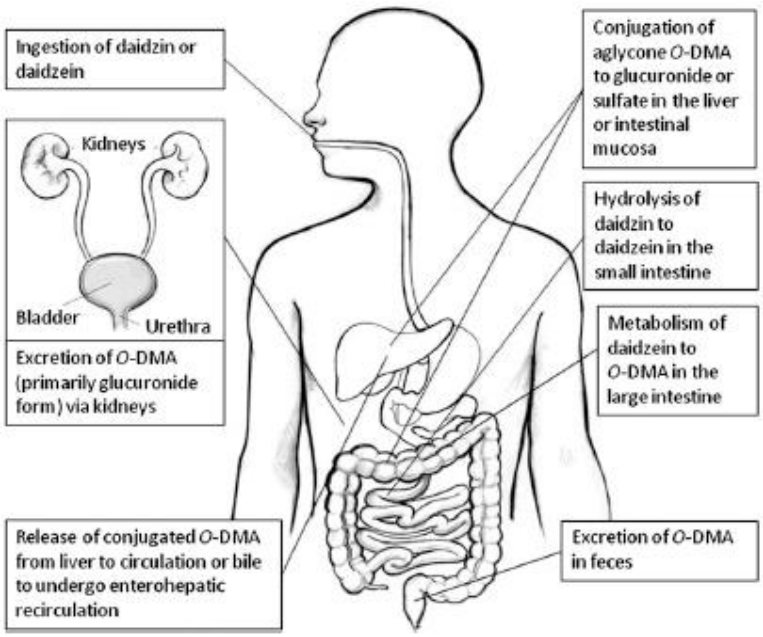


Figure 3: Production and metabolic fate of O-DMA.[3]

Considering the fact that equol-producing bacteria are distinct from *O*-DMA-producing bateria[3], soy isoflavones bacterial metabolism differs among individuals depending on the type of intestinal bacteria that exist in the GIT.[4] 80-90% of humans possess bacteria that are capable of producing *O*-DMA[4,6] and are referred to as *O*-DMA-producers[3], while only 30-50% possess equol-producing bacteria[4] and are referred to as equol-producers.[3]

Regarding *O*-DMA-producing bacteria, very few strains were identified to be able to cleave the C-ring of daidzein, which include *Eubacterium ramulus*, *Eubacterium ramulus* Julong 601 and *Clostridium* HGH 136.[7,8] *E. ramulus* represents approximately 0.16% of the total fecal flora, which is a comparable amount to that of *E. coli*, thus it can be considered as one of the major bacteria in the human GIT able to degrade isoflavonoids, though it was found that *E. ramulus* was not able to further metabolize *O*-DMA.[5] Recently, a novel strain SY8519 belonging to *Clostridium* rRNA cluster XIVa was isolated from human feces[6] and its complete genomic sequence was determined.[9] Not only was it able to metabolize daidzein, but also a variety of other polyphenols.[6] However, the genes associated with daidzein metabolism could not be predicted.[9]

1.1.3 Biological properties

Isoflavones and their metabolites, including *O*-DMA, have been observed to exert several biological actions in *in vitro* and in animal studies.[3,7] Having a diphenolic structural similarity to estradiol (E_2), they have been established to have a weak estrogenic or antiestrogenic activity.[10] Compared to the precursor isoflavone daidzein, other isoflavones and their metabolites, *O*-DMA demonstrated the strongest binding activity to hER β, as strong as E2, but a weaker binding activity to hER α.[13] Furthermore, it showed antiproliferative effects on human hepatocellular carcinoma (HCC) Hep3B cells with a lower IC_{50} value than that of daidzein. The mechanism of action appeared to occur via cell cycle arrest at the G_2/M phase and subsequent apoptosis. This occurred through reduction of CDK1, downregulation of Bcl-2 and upregulation of Bax, which lead to the mitochondrial release of cytochrome *c* and caspase-3 activation inducing apoptosis.[11] Another recent study supported these results by showing that *O*-DMA caused inhibition of cell proliferation and induction of apoptosis of human breast cancer MCF-7 cells through cell cycle arrest at G_1/S and G_2/M phases as well as increasing and decreasing the levels of the cell cycle regulators CDK4/6-cyclin D and CDK1-cyclin B complexes respectively, affecting their interactions.[12] All these findings confirm the hypothesis of *O*-DMA being a promising anticancer drug candidate.[11]

On a related aspect, a study conducted on 55 postmenopausal women demonstrated that the percent mammographic density, which is an intermediate marker of breast cancer risk, was 69% greater in *O*-DMA producers than non-producers, whilst 39% lower in equol producers than non-producers. Further adjustment for selected circulating hormone concentrations (FSH, free testosterone) suggested that these endogenous hormones are responsible for this relationship between mammographic density and *O*-DMA-producer phenotype. Nevertheless, the exact mechanism by which daidzein metabolites, equol and *O*-DMA, could affect the mammographic density is still not known. Though there are several hypotheses, including the binding capability of equol and *O*-DMA to estrogen receptors, exogenous and/or genetic factors influencing the intestinal environment allowing daidzein-metabolizing bacteria to flourish. This study had a serious limitation of utilizing only one sample of a small size, thus these results should be confirmed with a larger sample size and more diverse specifications.[14,15]

Ishimi *et al.* studied the effects of *O*-DMA on bone and lipid metabolism in OVX mice. They observed that bone loss was not affected as well as only weak inhibition of osteoclast formation *in vitro*. However, *O*-DMA did influence the plasma lipid profile, where decreased levels of TG, TC[18] and LDL and increased levels of HDL[17] were observed in estrogen-deficient mice[18] and postmenopausal women.[17] These effects could be due to ER binding in addition to inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, which is involved in cholesterol biosynthesis[18], though the exact mechanism should be further studied.

1.1.4 Chirality

Equol has been much studied and it was identified that the (*S*)-(-) enantiomer **3a** is the major form produced during intestinal bacterial metabolism of daidzein.[19,20] However, only recently, a couple of papers were published identifying the main enantiomeric form of the other optically active daidzein metabolite to be (*R*)-(-)-*O*-DMA **4a**.[7,21] Figure 4 shows the structures of the major enantiomeric forms of equol and *O*-DMA found in human plasma and urine post intestinal bacterial metabolism of daidzein. Time-Dependent Density-Functional Theory (TDDFT) calculations, together with Circular Dichroism (CD) spectra of pure *O*-DMA enantiomers in ethanol were used to establish the absolute configuration and the major form's optical rotation $[\alpha]_D^{25}$ was also measured and found to have a negative value (-135.8º).[7,21]

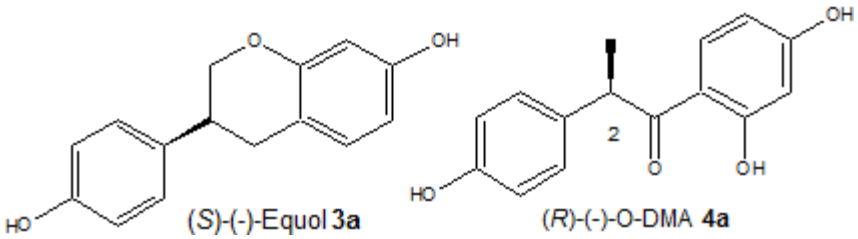


Figure 4: Structures of (S)-(-)-Equol and (R)-(-)-O-DMA.

1.1.5 Synthesis

There are various ways to obtain racemic *O*-DMA **4**. In literature, it was either provided by Plantech (Reading, UK)[7] or more commonly synthesized by a one-step Friedel-Crafts acylation of resorcinol **6** with racemic 2-(4-hydroxyphenyl)-propionic acid **7** in $BF_3.Et_2O$ (Figure 5).[21-23] A study conducted to investigate the anticancer effects of *O*-DMA prepared it using a different multistep synthetic route (Figure 6).[11] Both protocols claimed to produce similar yields of approximately 80-90%, thus the Friedel-Crafts acylation seems to be much simpler, faster and cheaper.

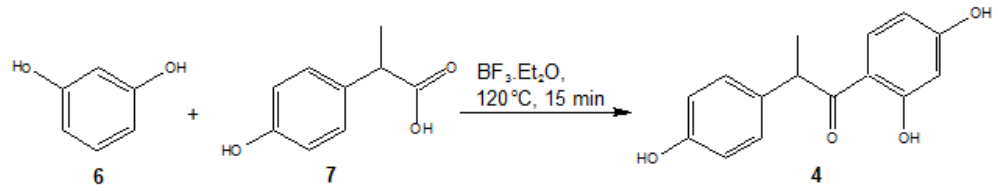


Figure 5: Synthesis of racemic O-DMA by Friedel-Crafts acylation (80% yield[22,23]).

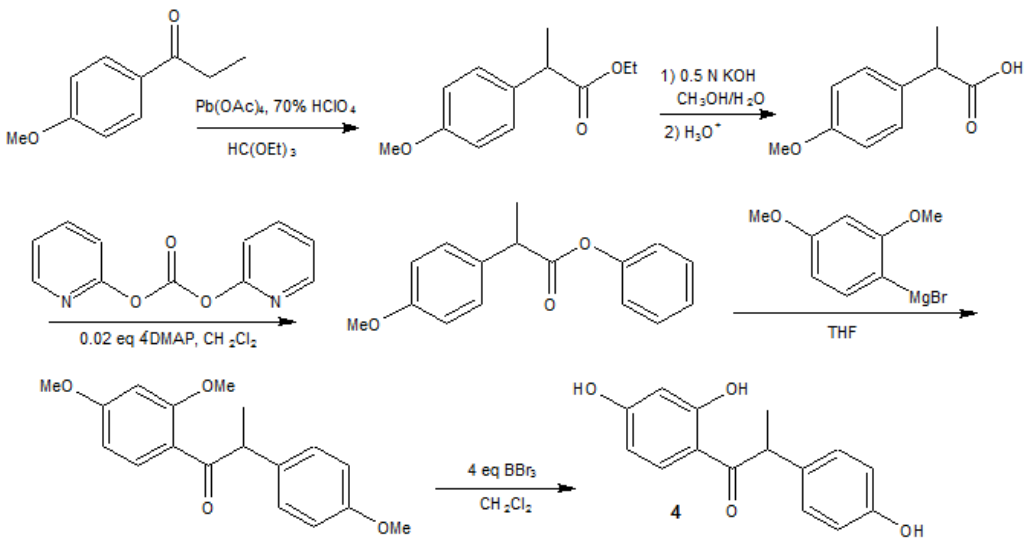


Figure 6: Alternative longer route for racemic O-DMA synthesis (90% yield[11]).

To date, the sole method used to obtain enantiopure *O*-DMA is through preparative chiral HPLC separation. A Sumi-chiral column, OA 7000 (8x250mm, Sumika Chemical Ltd., Osaka, Japan) is used and separation is done using isocratic 40% acetonitrile in water.[7,8,21]

1.2 Asymmetric synthesis of 2-phenylpropanoic acid

Given that the chirality of *O*-DMA **4a** exists at C-2, which originates from the acid component **7a** of the Friedel-Crafts acylation, it was essential to review the different methods reported in literature for the synthesis of enantiopure 2-arylpropanoic acid. Aside from the use of a chiral auxiliary for asymmetric *α*-alkylation, which will be explained further in the next chapter, various catalytic approaches were studied for the asymmetric synthesis of 2-phenylpropanoic acid **8** (Figure 7) as a model compound. The most common method is homogenous catalysis using transition metals coordinated with chiral ligands, but also some electrochemical and enzymatic catalysis were reported.

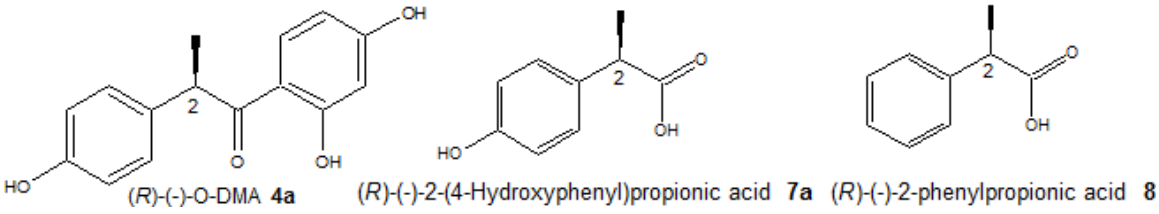


Figure 7: Structures of (R)-(-)-O-DMA, its acid component ((R)-(-)-2-(4-hydroxyphenyl)propionic acid) and (R)-2-phenylpropionic acid.

1.2.1 Asymmetric hydrogenation

One of the most studied reactions of homogenous catalysis is the asymmetric hydrogenation of olefins using transition metal complexes, most commonly of Ru(II)[24,25,27,28,32], Ir(I)[29,31] and Rh(I).[26,30,33] This can be utilized for the conversion of 2-phenylacrylic acid **9** to enantiopure (*R*)-2-phenylpropanoic acid **8** (Figure 8).

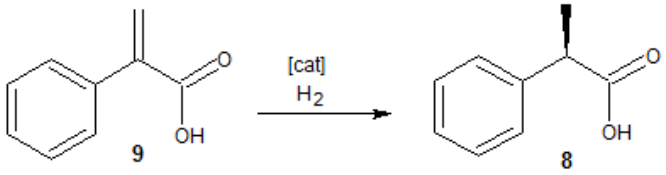


Figure 8: Asymmetric hydrogenation of 2-phenylacrylic acid.

For the past 20 years, several chiral phosphine ligands have been developed and their reaction conditions have been studied to improve the results. The highest yields (>90 %) and % ee (96-

97%) were achieved using chiral ligands of cyclic nature; *P*-stereogenic phosphine ligands with Rh(I)[30] **16**, **17** (entries 7 and 8, Table 1), SpinPHOX ligands[29] **15** (entry 6, Table 1) and spiro aminophosphine ligands[31] with Ir(I) **18** (entry 9, Table 1). Also, attempts to improve the product separation and catalyst recovery have been investigated, which achieved 86-92% ee. Examples include the use of room temperature molten salts, as $BMI.BF_4$[24], (entry 1, Table 1) or liquid PEGs[28] (entry 5, Table 1) in the solvent system and dendritic chiral ligands[32] **19** (entry 10, Table 1) to immobilize the catalytic transition metal complex. Other types of chiral phosphine ligands were employed such as: biheteroaromatic (-)-tetra-Me-BITIOP[2] **11** (entry 2, Table 1), ferrocene-based 1,3-bis(phosphanes) with planar chirality[26] **12** (entry 3, table) and monodentate secondary phosphine oxide[33] (SPO) **20** (entry 11, Table 1). Table 1 summarizes some of the transition metal complexes used as catalysts for the asymmetric hydrogenation of 2-phenylacrylic acid along with the solvent(s) used, reaction conditions [S/C (substrate/catalyst ratio), P_{H2} (H_2 pressure), T (temperature)], % yield, % ee and the conformation (conf.) of the major enantiomer formed. Figure 9 shows the structures of the transition metal complexes and/or ligands listed in Table 1.

Table 1: Transition metal complexes used for the asymmetric hydrogenation of 2-phenylacrylic acid

Entry	TM complex	Solvent(s) (ratio)	S/C	P_{H2} (atm)	T (°C)	Yield (%)	% ee (conf.)
1	Ru-(*R*)-BINAP[24] **10**	MeOH/BMI.BF_4	40	25	rt	-	86 (*R*)
2	(-)-[(TetraMe-BITIOP)Ru Bis(2-methallyl)][25] **11**	MeOH	160	50	20	-	94 (*R*)
3	[Rh(cod)Fe/bis(phosphane)]PF_6[26] **12**	MeOH	-	1	-	90	83 (*R*)
4	(*R*,*R*)-TsDPEN-Ru(II)[27] **13**	DCM	100	-	30	97	7 (*R*)
5	PEG supported Ru-(*R*)-BINAP[28] **14**	PEG-600/MeOH (1:2)	100	50	20	-	92.4 (*R*)
6	Ir-(*R*,*S*)-SpinPHOX[29] **15**	MeOH	100	30	50	-	96 (*R*)
7	[Rh((*R*,*R*)-cPen-SMS-phos)$(MeOH)_2$]BF_4[30] **16**	MeOH	100	Ambient	rt	>90	96.4 (*R*)
8	[Rh((*R*,*R*)-Cy-SMS-phos)$(MeOH)_2$]BF_4[30] **17**	MeOH	100	Ambient	rt	>90	97.1 (*S*)
9	Ir complex with chiral spiro aminophosphine ligands[31] **18**	MeOH	1000	6 (15min) Ambient (4h)	45	97 95	98 (*R*) 98 (*R*)
10	[Ru(*p*-cymene)Cl_2]$_2$/ dendritic BINAP[32] **19**	MeOH/toluene (1:1)	-	80	20	-	86 (*R*)
11	[Rh(μ-Cl)(cod)]$_2$/(*S*,*S*)-SPO[33] **20**	Dioxane	200	30	rt	-	93 (*R*)

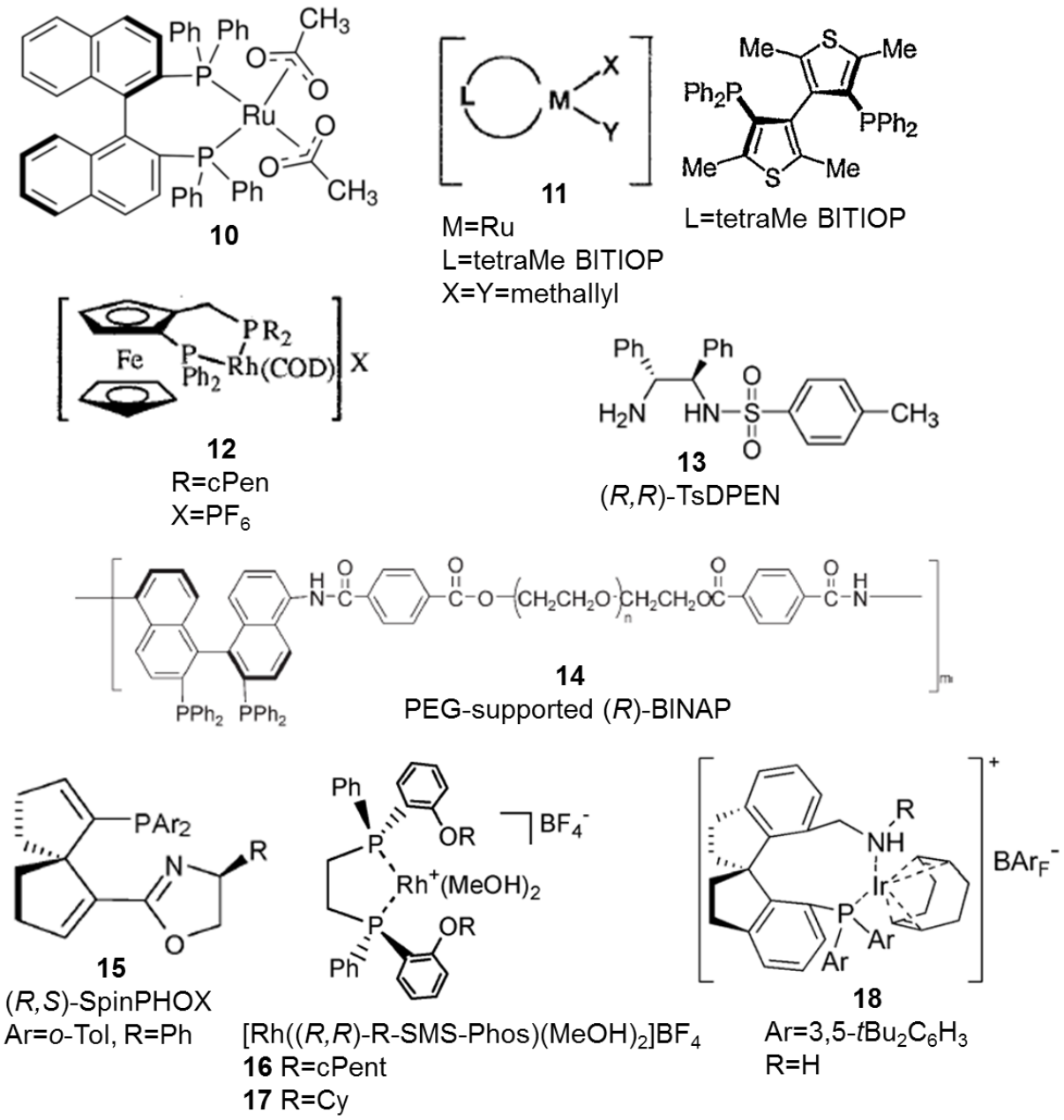
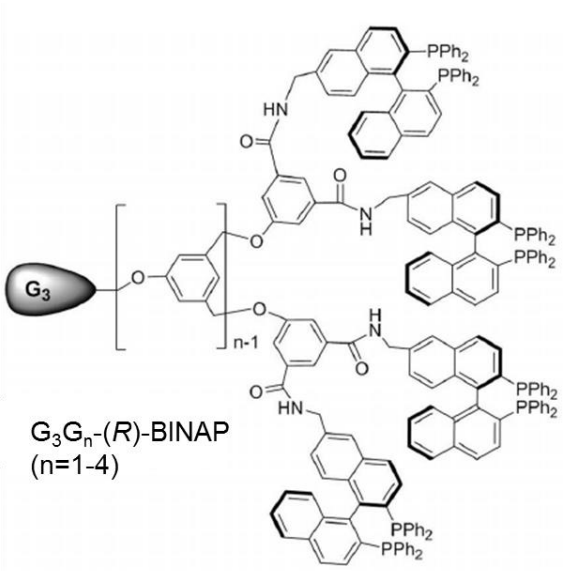
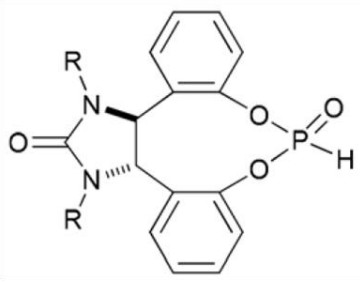


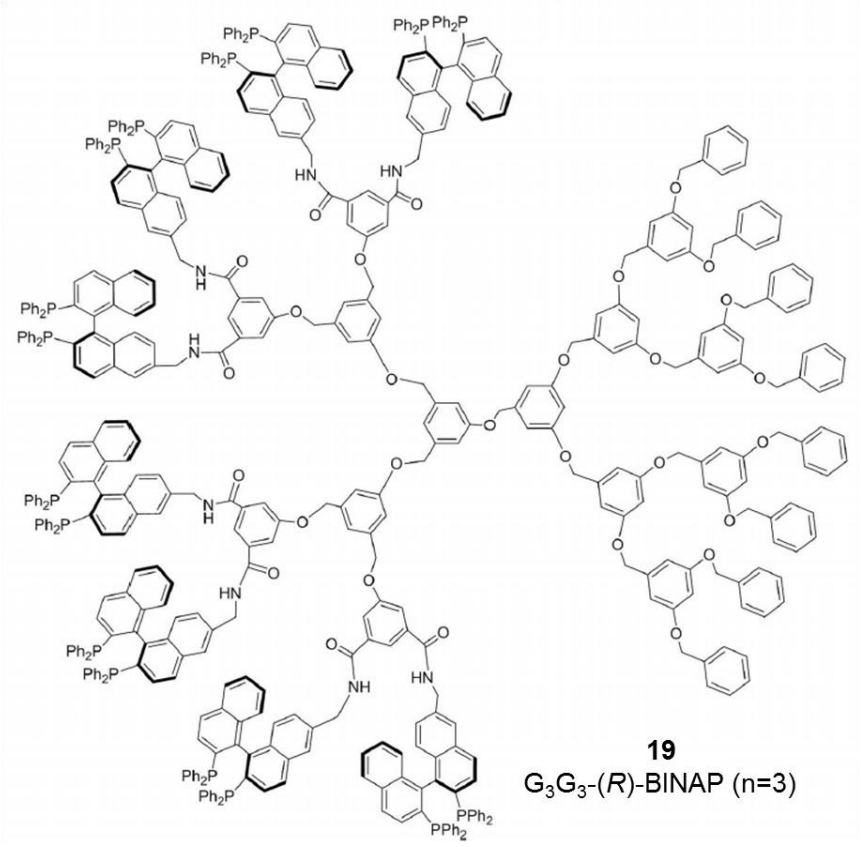
Figure 9: Structures of the transition metal complexes or ligands listed in Table 1



G_3G_n-(*R*)-BINAP
(n=1-4)



20
(*S*,*S*)-SPO
R=Bn



19
G_3G_3-(*R*)-BINAP (n=3)

Figure 9 cont.: Structures of the transition metal complexes or ligands listed in Table 1

1.2.2 Asymmetric hydrocarboxylation

Another type of functionalization of olefins is asymmetric hydrocarboxylation, which can be done using homogenous catalysis, where, as a model relevant to the purpose of this work, styrene **21** can be converted to enantiopure (*R*)-2-phenylpropanoic acid **8** (Figure 10).[34-38]

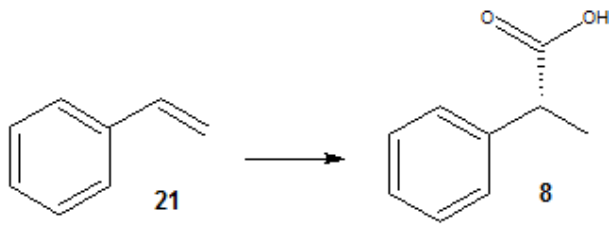


Figure 10: Asymmetric hydrocarboxylation of styrene.

In literature, there are a few approaches to which how this can be achieved. The most direct route is the use of CO and H_2O in the presence of a transition metal chiral complex. However, the linear carboxylic acid product **23** will be partly formed along with the desired branched product **8** (Figure 11). Earlier research using Pd with chiral sulfonated diphosphine ligands, (*S*,*S*)-BDPPTS **25** and (*R*,*R*)-CBDTS **26**, yielded low branched/linear (b/l) acid products ratio (0.52 and 0.41 respectively) with a %ee of only 32% (*R*) and 14% (*S*) respectively (entries 5 and 6, Table 2).[36] Further studies using dipalladium complexes with chiral phanephos ligands **27**[37] and **28**[38] improved the b/l ratio in favor of the branched product with approximately 80% ee (*R*) (entries 7 and 8, Table 2), where in the latter case, MeOH was used instead of H_2O and the branched product is formed almost exclusively (b/l >100).[38]

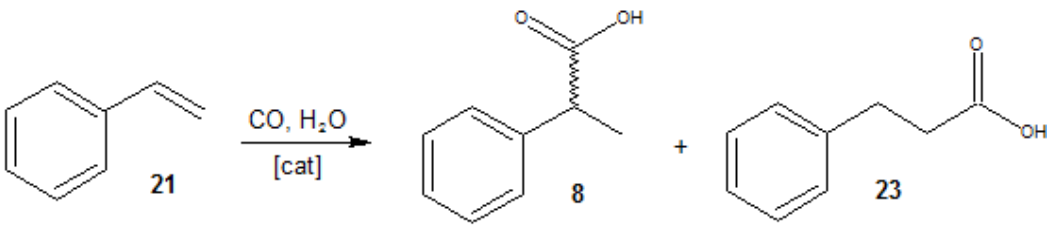


Figure 11: Asymmetric hydrocarboxylation of styrene using CO and H_2O.[34-38]

An alternative strategy is to separate the metal hydride addition step creating the stereocenter followed by the stereospecific C-C bond formation. In this case, a stable carbon surrogate (such as: boron), which can be easily replaced by carbon in a stereospecific manner, is required. Asymmetric catalytic hydroboration of styrene followed by homologation with $LiCHCl_2$ (generated by deprotonation of DCM with *n*-BuLi) and oxidation with $NaClO_2$ (Figure 12) was found to be successful in producing (*R*)-2-phenylpropanoic acid **8** with moderate yields (45-87%) and 93-95% ee (entries 1-4, Table 2).[34,35]

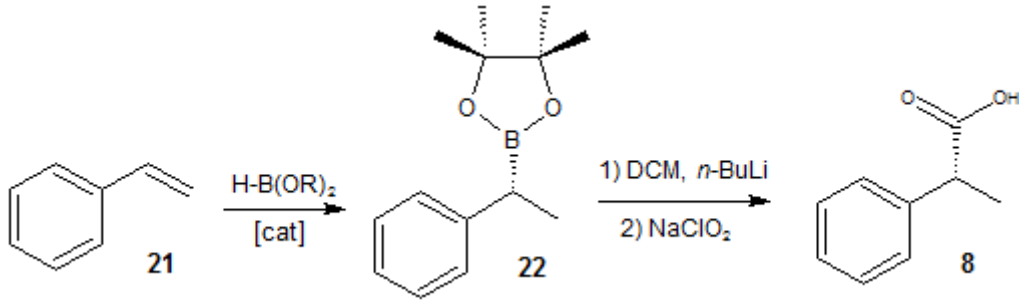


Figure 12: 2-Step asymmetric hydrocarboxylation of styrene.[34,35]

Asymmetric catalytic hydroformylation of styrene with CO and H_2 followed by oxidation of the aldehyde moiety into a carboxylic acid can also be another synthetic scheme. A Rh(I) complex with glucopyranoside-based diphosphite ligand **29** was reported to yield 2.85 b/l ratio and 41% ee (entry 9, Table 2).[39] Table 2 summarizes some of the transition metal complexes used as catalysts for the asymmetric hydrocarboxylation of styrene along with the catalyst and reagents used, b/l ratio, % yield, % ee and the conformation (conf.) of the major enantiomer formed. Figure 13 shows the structures of the transition metal complexes and/or ligands listed in Table 2.

Table 2: Transition metal complexes used for the asymmetric hydrocarboxylation of styrene

Entry	Catalyst	Reagent	b/l	Yield (%)	% ee (conf.)
1	$[Rh(cod)_2]BF_4$/(*R*)-BINAP[34] **24**	$(RO)_2BH$	-	95	95 (*R*)
2		1. $LiCHCl_2$, $ZnCl_2$ 2. $NaClO_2$	-	45	95 (*R*)
3	$[Rh(cod)_2]BF_4$/(*R*)-BINAP[35] **24**	$(RO)_2BH$	-	99	93 (*R*)
4		1. $LiCHCl_2$ 2. $NaClO_2$	-	87	93 (*R*)
5	$Pd(OAc)_2$/(*S*,*S*)-BDPPTS[36] **25**	CO (20 bar), H_2O	0.52	-	32 (*S*)
6	$Pd(OAc)_2$/(*R*,*R*)-CBDTS[36] **26**	CO (20 bar), H_2O	0.41	-	14 (*R*)
7	Dimetallic Pd/(*R*)-phanephos ligands complex[37] **27**	CO (30 bar), H_2O	1.1	71	80 (*R*)
8	Dimetallic Pd/(*R*)-phanephos ligands complex[38] **28**	CO (30 bar), MeOH	>100	>99	79 (*R*)
9	$Rh(acac)(CO)_2$/(*S*)-binaphthol[39] **29**	1. CO (3 bar), H_2 (17 bar) 2. Jones oxidation	2.85		41 (*S*)

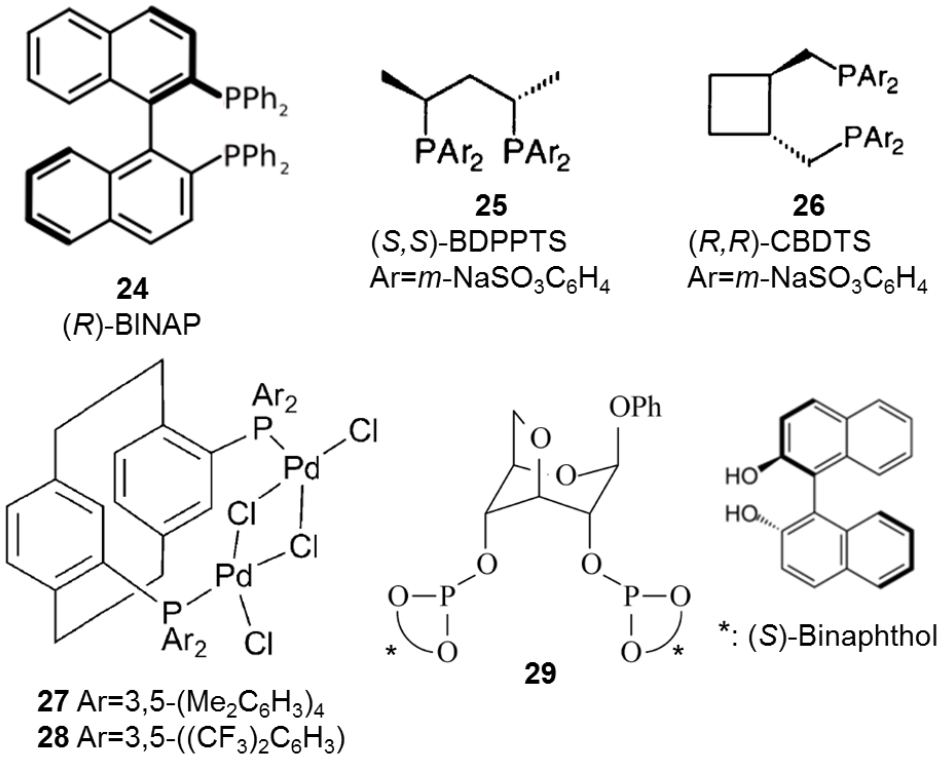


Figure 13: Structures of the transition metal complexes or ligands listed in Table 2

1.2.3 Asymmetric electrocarboxylation

Aside from homogenous catalysis, the carboxylic acid functional group can also be added using electrochemistry. In the past decades, electrochemical reduction of aromatic halides in CO_2-saturated solutions for the synthesis of their corresponding carboxylic acids has been of much interest as an alternative to the use of homogenous catalysis.[40] However, very few studies have been done concerning asymmetric electrocarboxylation. Feroci *et al.* have been successful to synthesize chiral methylmalonic ester[41,42] and cinnamic acid derivatives.[43] Recently, electrochemical asymmetric carboxylation of phenylethyl chloride **30** with CO_2 using chiral Co^{II}-(*R,R*)(salen) **31** (Figure 15) as a catalyst to produce (*R*)-2-phenylpropionic acid **8** was reported (Figure 14).[44] The best results were of 37% yield and 83% ee (*R*), which were obtained in solvent DMF at the optimum conditions: temperature of 50ºC, charge of 3.0 $Fmol^{-1}$, substrate concentration of 50 mM, catalyst/substrate ratio of 15 mol%, applied potential of (E_{app}) of -1.59 V and CO_2 pressure (P_{CO2}) of ~ 1 atm ($1x10^5$ Pa). They also stated that the reason for the low yield values is probably due to reduction of intermediates supported

by the GC-MS detection of unreacted benzyl chloride, 2,3-diphenylbutane, phenylethane and styrene. Nevertheless, further research in this asymmetric electrocarboxylation system could provide a new synthetic route of enantiopure chiral carboxylic acids from achiral substrates.[44]

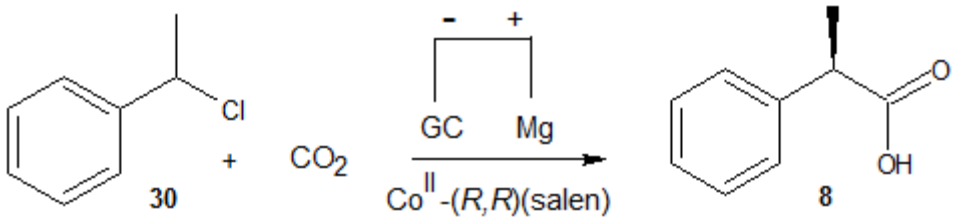


Figure 14: Asymmetric electrocarboxylation of phenylethyl chloride.[44]

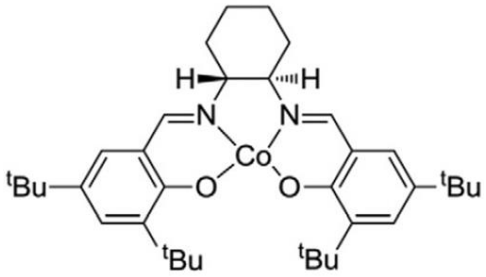


Figure 15: Structure of the chiral catalyst Co^{II}-(R,R)(salen).[44]

1.3 Asymmetric α-alkylation

1.3.1 Chiral auxiliary

Asymmetric *α*-alkylation of carboxylic acid derivatives is a useful reaction in organic synthesis. These *α*-alkylated derivatives are versatile synthetic intermediates; where the carboxylic acid moiety can undergo a variety of transformations. An effective method to achieve this transformation is based on the use of a chiral auxiliary. Over the years, a lot of work has been done to design new chiral auxiliaries with more efficient yields and better optical purities.[52]

Chiral auxiliary-based asymmetric alkylation of carboxylic acid enolate was first reported by Myers *et al.* in 1976, where oxazoline anions were readily alkylated by a variety of alkyl halides and showed high diastereoselectivities.[45] Evans *et al.* first described the asymmetric alkylation of N-acyl oxazolidinones **32** in 1982 (entry 1, Table 3) (Figure 16). Further improvements were done using Ti-enolates **33** instead of Li- or Na-enolates (entry 2, Table 3) (Figure 17).[52] Moreover, *S*-proline was successfully used as a chiral auxiliary in asymmetric *α*-alkylation of phenyl acetic acid to give (*R*)-2-phenylpropionic acid **8** ($[\alpha]_D^{25}$ = -76º) in 65% yield and 80% de of the alkylated intermediate **34a** (entry 3, Table 3) (Figure 18).[53] Several camphor-based chiral auxiliaries[52,54] (Figures 19 and 20) have also been reported for the asymmetric *α*-alkylation of carboxylic acids with high yields and diastereoselectivities (entries 4 and 5, Table 3). Another very common chiral auxiliary studied for this type of transformation is pseudoephedrine; which will be explained in more details later. Table 3 summarizes the different chiral auxiliaries, aside from pseudoephedrine, explained above along with the reaction conditions and results of some of their alkylation reactions.

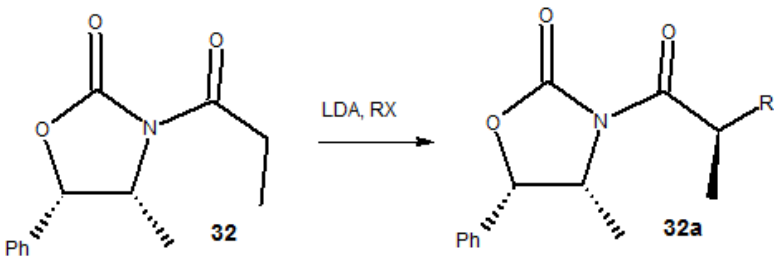


Figure 16: Oxazolidinone-based asymmetric alkylation.[52]

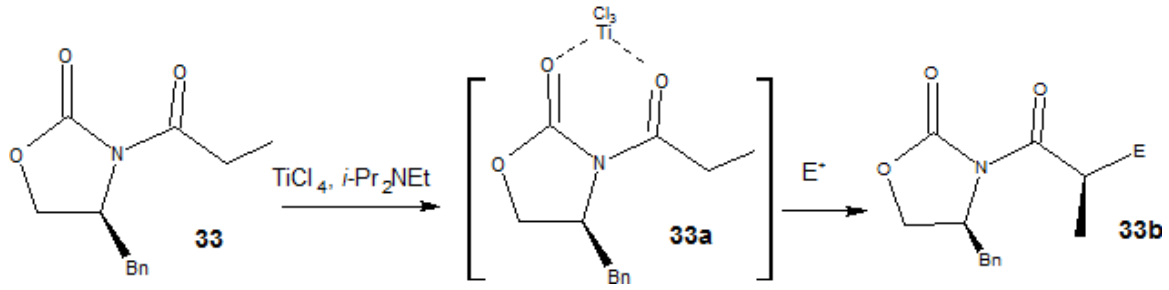


Figure 17: Oxazolidinone-based asymmetric alkylation via Ti-enolates.[52]

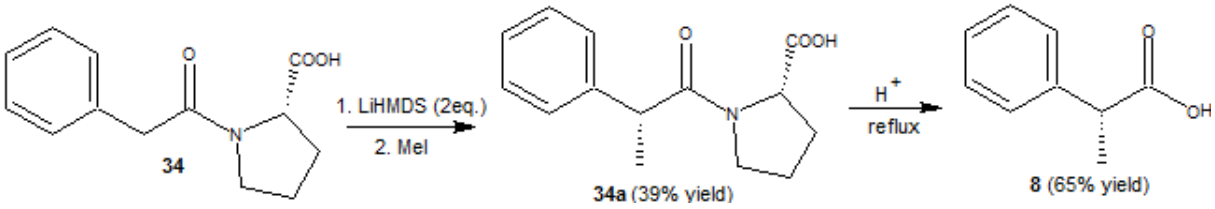


Figure 18: S-proline-based asymmetric alkylation.[53]

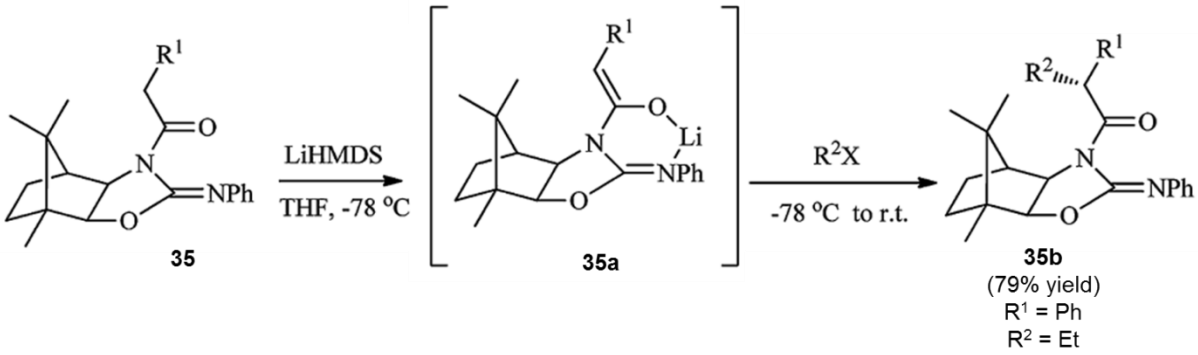


Figure 19: Camphor-based 2-phenylimino-2-oxazolidine chiral auxiliary.[54]

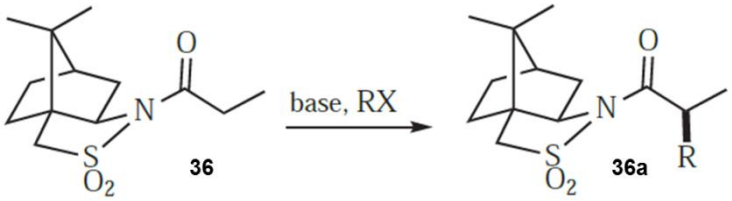


Figure 20: Camphor-based Oppolzer sultam chiral axiliary.[52]

Table 3: Chiral auxiliaries used for asymmetric α-alkylation of carboxylic acids

Entry	Chiral auxiliary	Base	RX/E	Alkylated intermediate	% de
1	Oxazolidinone[52]	LDA	BnBr EtI	**32a**	98 88
2	Oxazolidinone[52]	$TiCl_4$, *i*-Pr_2NEt	$BnOCH_2Cl$	**33b**	>100
3	(*S*)-Proline[53]	LiHMDS	MeI	**34a**	80
4	2-Phenylimino-2-oxazolidine[54]	LiHMDS	EtI	**35b**	>99
5	Oppolzer sultam[52]	NaHMDS	BnI	**36a**	98

1.3.2 Pseudoephedrine as chiral auxiliary

1.3.2.1 Advantages

The key advantages of using pseudoephedrine as chiral auxiliary depend mainly on the fact that it is inexpensive and easily available in bulk in both enantioforms.[47] Moreover, they are easily acylated to form their amides, which are usually crystalline[46,48] and can undergo a variety of diastereoselective reactions in a highly efficient manner including alkylation reactions using many alkyl halides.[46] In addition to its synthetic versatility producing interesting chiral building blocks with different functional groups[47,49], the pseudoephedrine moiety can be easily removed and recovered without considerable loss providing a further benefit of recyclability.[47,50]

1.3.2.2 Synthesis of pseudoephedrine amides

Formation of amide bond is a fundamental and established reaction in organic chemistry. *N*-acylation of the amino alcohol pseudoephedrine **37** is an example of this transformation. As shown in Figure 21 below, the carboxylic acid moiety should be activated in order for the acylation reaction to proceed in a high yield. The carboxylic acid derivatives most commonly used are anhydrides or acid chlorides. While the carboxylic acid anhydrides can undergo

efficient acylation in DCM or THF as solvent and the presence of base is not essential other than to fasten the reaction, acylation with carboxylic acid chlorides needs the addition of a slight excess of base, such as: Et_3N. Nevertheless, they readily react at low temperatures (0 ºC) in most organic solvents. If neither the anhydride nor the acid chloride is accessible, mixed anhydride from the carboxylic acid, pivaloyl chloride and Et_3N was found to be a suitable alternative.[46]

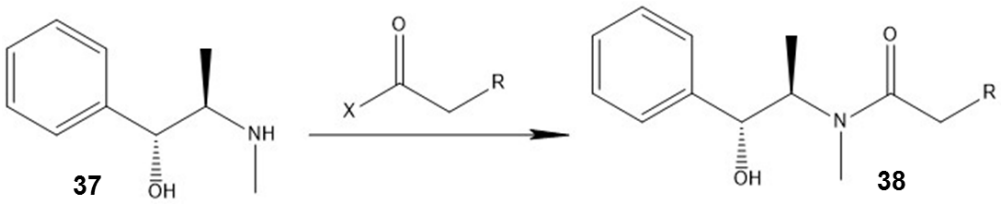


Figure 21: N-acylation of pseudoephedrine[46]

The formed amide products **38** are anhydrous, air-stable and highly crystalline. A small amount of *N*,*O*-diacylated compound was the only byproduct reported, which can be easily separated by direct recrystallization of the crude product or by flash chromatography.[46]

1.3.2.3 Asymmetric alkylation of pseudoephedrine amides

As mentioned above, pseudoephedrine amides **34** undergo efficient asymmetric α-alkylation reactions using alkyl halides, which can be attributed to the fact that the amide functionality is highly reactive and its enolate is a strong chiral nucleophile.[50,51] There are two options to carry out this diastereoselective alkylation reaction: either using excess alkyl halide or excess enolate. In the former case, the yield will be dependent on the enolate (limiting reagent) and vice versa in the latter case. It has been reported that the yield is slightly higher when the alkyl halide is the limiting reagent. However, it is not significant enough and the choice is usually based on the availability and the cost of one reagent relative to the other. Enolization is usually done by the base, LDA, which is formed by mixing in dry THF *n*-DIPA with *n*-BuLi in hexanes at -78 ºC. In both cases, reverse addition of starting materials is employed, where the pseudoephedrine amide solution is added gradually to a cold suspension of LDA/LiCl to produce the enolate followed by addition of the alkylating agent (Figure 22).[46]

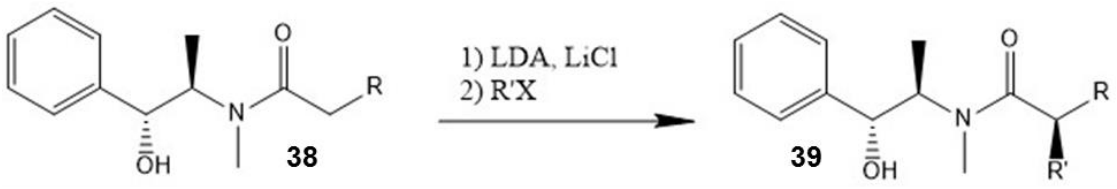


Figure 22: Diastereoselective alkylation of pseudoephedrine amides[46]

The reason for adding lithium chloride (LiCl) to the reaction mixture is to speed up the rate of alkylation as well as to suppress *O*-alkylation of the pseudoephedrine secondary alcohol group, thus decreasing the amount of byproducts. Myers et al. recommended the addition of 6.0-7.0 equiv. of LiCl and not less than 4.0 equiv. for optimum reaction rate and yield. They also proved that the presence of LiCl does not influence the diastereoselectivity of the alkylation reaction. However, it should be taken as a precaution that the LiCl used is completely anhydrous because if any water is present, this may lead to quenching the base (LDA) and enolization will not be successful.[46]

Pseudoephedrine amide enolates **34a** are highly reactive nucleophiles, therefore they react efficiently with primary alkyl halides at a temperature as low as -78 ºC and with high diastereoselectivity. The major alkylated product **35** is the result of the electrophilic attack of the alkyl halide on the (*Z*)-enolate from the same face as the methyl group of the pseudoephedrine, i.e. the additional alkyl group and the pseudoephedrine methyl group are on the same side of the molecule (Figure 23). When using β-branched alkyl halides, they also react efficiently, but a higher temperature is needed (23 ºC), which does not cause a problem due to the thermal stability of the pseudoephedrine amides.[46]

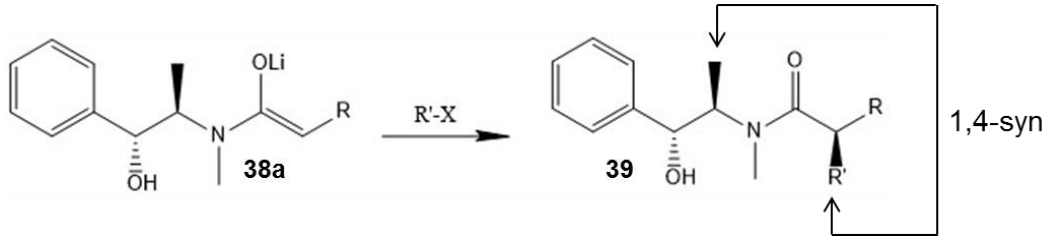


Figure 23: Pseudoephedrine amide enolate alkylation[46]

1.3.2.4 Hydrolysis of pseudoephedrine amides

To form carboxylic acids **36** from the alkylated products **35** of pseudoephedrine amides, hydrolysis can be achieved in acidic or basic conditions. If the compounds are not acid-sensitive, refluxing in a 1:1 mixture of concentrated sulfuric acid and dioxane will yield the corresponding carboxylic acid product. If these conditions are too harsh and decomposition or significant decrease in the product yield or optical purity occur, lower concentrations of sulfuric acid can be used, though longer times will be required (Figure 24).[46]

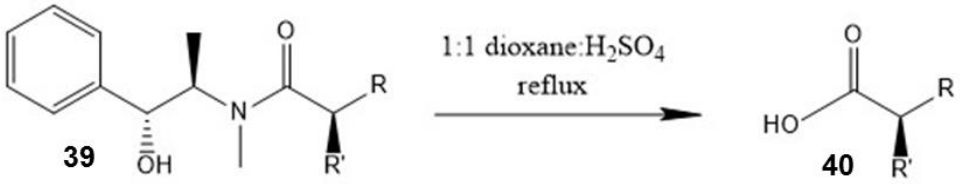


Figure 24: Acidic hydrolysis of pseudoephedrine amides[46]

An alternative method for hydrolysis of acid-sensitive compounds was developed using a variety of hydroxide bases in different solvent systems. An example that was found optimal is using 5 equiv. of tetra-*n*-butylammonium hydroxide in a 1:4 mixture of *t*-butanol and water at reflux (Figure 25). In either cases of acidic or basic hydrolysis, the pseudoephedrine chiral auxiliary can be recovered by a simple extraction step during work-up.[46]

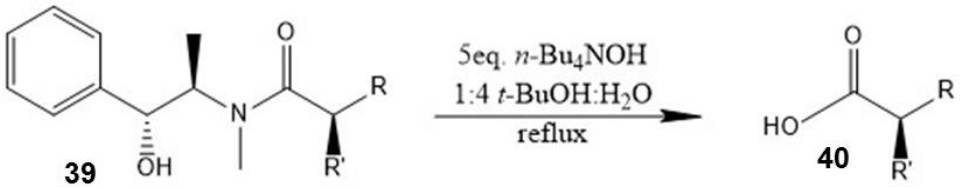


Figure 25: Basic hydrolysis of pseudoephedrine amides[46]

Lewis acidic metal salts such as: $ZnCl_2$, $FeCl_3$, $ZrOCl_2$ and $Yb(OTf)_3$ were studied to develop another hydrolysis procedure using milder conditions. Increasing the ratio of water:dioxane solvent system from 1:1 to 4:1 lead to increasing the yield from an average of 90% to 94%. However, the long reaction times required in these types of metal-mediated reactions (48hrs) limit their use and encourage the search for other milder hydrolysis methods.[46]

1.3.3 Chiral lithium amide bases

In 2011, Stivala and Zakarian developed an alternative single-step method for asymmetric alkylation of arylacetic acid, first recognized by Shioiri and Koga more than a couple of decades ago. Instead of using a chiral auxiliary, they employed chiral lithium amides as non-covalent stereodirecting agents. The alkylation reaction proceeds via enediolates **41a** as reactive intermediates (Figure 26), which are of high nucleophilicity and limit the *E/Z* selectivity issues during enolization due to their geometrical symmetry. Several C_2-symmetric tetramines were tested and the best enantioselectivity (93% ee, Figure 26) was achieved with a tetramine containing piperidine subunits and three-carbon linker (Figure 27). Optimum conditions used were 4.0 eq. of *n*-BuLi and slight excess (1.03 eq.) of chiral tetramine **42**, which was recovered by extraction with aqueous HCl. It was found that enantioselectivity depended on the quality of *n*-BuLi, where lower % ee (52%) was observed when *n*-BuOLi, an impurity commonly found in aged *n*-BuLi bottles, was added to the reaction mixture. Moreover, methylation with more hindered alkyl halides, such as: isobutyl (98% ee), isopropyl (97% ee), and cyclopentyl iodides (96% ee), gave higher enantioselectivities compared to the smaller methyl iodide (88% ee), though they required longer reaction times.[55]

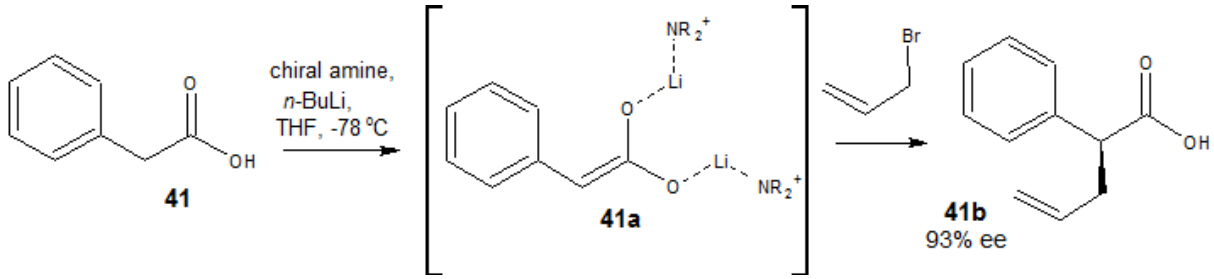


Figure 26: Direct asymmetric α-alkylation using chiral lithium amine.[55]

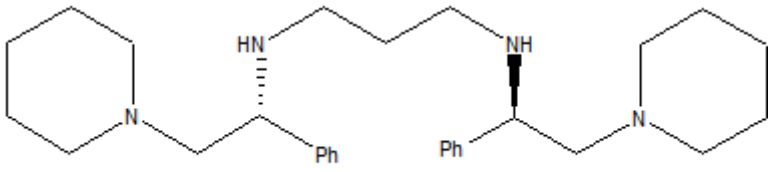


Figure 27: Structure of C_2-symmetric chiral tetramine.[55]

2 Aim of the study

The aim of this study was to develop a synthetic route of enantiopure (*R*)-(-)-*O*-desmethylangolensin. (*R*,*R*)-(-)-Pseudoephedrine was used as a chiral auxiliary, where the intermediate amide underwent stereoselective α-methylation. Figure 28 below outlines the synthetic route adopted in this work.

All steps, along with the successes and limitations are discussed. MS, IR and NMR spectroscopy were used to follow the reaction series progress.

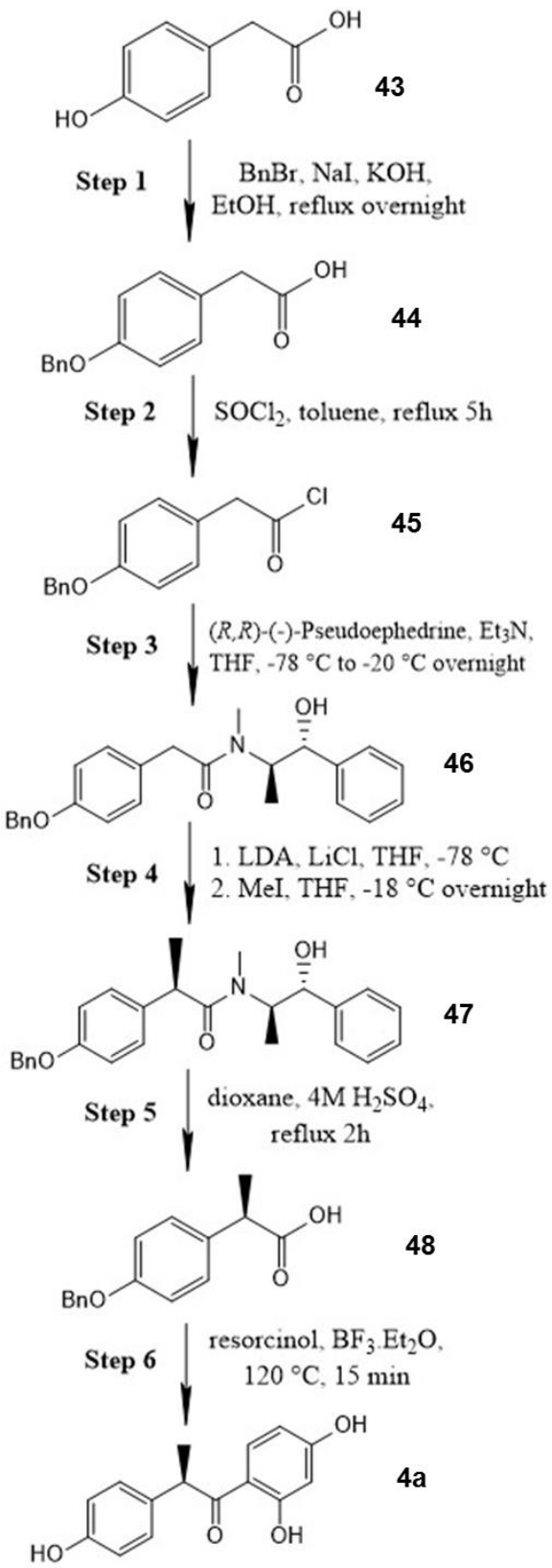


Figure 28: Synthetic route outline of (R)-(-)-O-DMA

3 Experimental Procedures

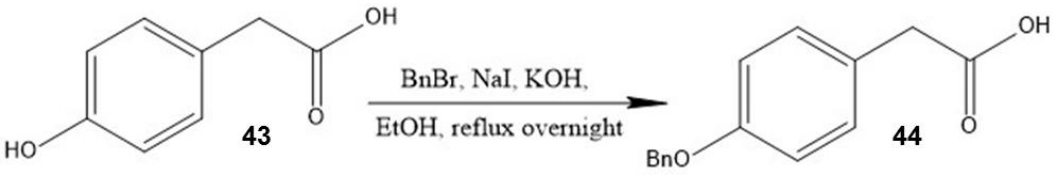
3.1 General materials

All EtOAc used in the work of this thesis was purchased from Sigma-Aldrich, DCM from Fisher Science, Et_2O (anhydrous) from J.T. Baker, n-hexane from Merck and MeOH from VWR Prolabo Chemicals. TLC experiments were done on Silica gel on TLC Al foils (254 nm) from Sigma-Aldrich. All Na_2SO_4 (anhydrous) used was from Merck. For NMR analyses, acetone-*d6* and $CDCl_3$ used were purchased from Eurisotop. All dry THF was dispensed from VAC solvent purifier.

3.2 Equipment

The rotary evaporator used in all experiments was R-210 from Büchi. ^{1}H and ^{13}C NMR analyses were done using Oxford 300 MHz from Varian. EI-MS analyses were done using JEOL JMS-700 MStation. IR spectra were taken using ALPHA FT-IR Spectrometer from Bruker. Whenever the product is solid, the melting point was measured by a Büchi Melting Point B-545 instrument. The specific optical rotation measurements were performed using DIP-1000 Digital Polarimeter from JASCO.

3.3 Step 1 [249RH81]



Scheme 1: Step 1 (benzyl protection)

3.3.1 Materials

EtOH (99.5%) was purchased from Etax. 4-hydroxyphenylacetic acid **43** was purchased from TCI Europe and benzyl bromide was purchased from Fluka. NaI and KOH were purchased from Merck. Concentrated H_2SO_4 (95-97%) was purchased from J. T. Baker.

3.3.2 Method

4-Hydroxyphenylacetic acid **43** (6.00g, 1eq.), KOH (5.53g, 2.5eq.) and NaI (0.12g, cat.) were weighed into a dry 500ml 2-neck round-bottom flask. 99.5% EtOH (240ml) and BnBr (5.15ml, 1.1eq.) were then added. A stirring bar was added and a septum was used to close off one neck of the flask. A reflux condenser was attached to the other neck after being flushed with Ar and a $CaCl_2$ tube put over the condenser. The mixture was left to reflux overnight in an oil bath while stirring. The next day, the reaction was monitored with TLC (7:2 DCM:EtOAc) to make sure there was no starting material left. The flask was taken out of the oil bath and left to cool down. The reaction was stopped by evaporating to dryness with the rotary evaporator. White solid was formed, to which distilled water (240ml) was added and the pH was measured (pH=8) using a pH paper. Concentrated H_2SO_4 (95-97%) was added carefully till the pH became below 7 and a white solid was formed. The mixture was then filtered under vacuum using a Büchner filter and washed with distilled water. The white solid was left to dry under vacuum for a few hours, then was transferred to a dry 100ml round-bottom flask to be dried in an oil pump for a few more hours and then overnight in the desiccator. Table 4 shows the % yields of step 1 trials performed.

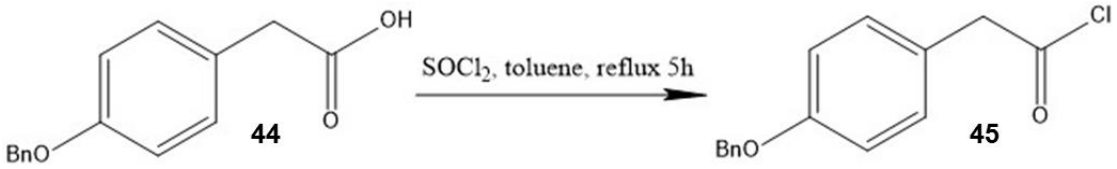
Table 4: %Yields of step 1 trials

Trial	% Yield
1 [249RH49]	91.4
2 [249RH81]	92

44 [249RH81]:

Yield after drying: 92%; white powder; melting point: 119-124 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.419 (2H), 7.394 (1H), 7.361 (m, 2H), 7.205 (d, 2H, *J* = 9 Hz), 6.955 (d, 2H, *J* = 9 Hz), 5.060 (s, 2H), 3.594 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.290, 158.318, 137.156, 130.647 (2C), 128.790 (2C), 128.170, 127.654 (2C), 125.805, 115.236 (2C), 70.254, 40.357; EI-MS *m/z* 242 ($[M+H]^+$), 212, 196, 121, 106, 91; IR 2400-3300 (carboxylic acid OH str.), 2905 (aliphatic CH str.), 1687 (acid C=O str.), 1510, 1244, 1012.

3.4 Step 2 [249RH83]



Scheme 2: Step 2 (acid chloride formation)

3.4.1 Materials

Thionyl chloride was purchased from Sigma-Aldrich. Toluene was purchased from Lab Scan Analytical Sciences. Both were distilled in Ar atmosphere immediately before use.

3.4.2 Method

Product from step 1 [249RH81] **44** (5.03g, 1eq.) was weighed into a dry 100ml 2-neck round-bottom flask with a magnetic stirrer and a rubber septum. Dry distilled toluene (75ml) was added to dissolve the solid under Ar atmosphere (using Ar balloon) and left in an ice bath to cool to 0 ºC. Dry distilled thionyl chloride (3.9ml, 1.28eq.) was then carefully added at 0 ºC

through the septum followed by attaching reflux condenser with $CaCl_2$ tube after being flushed with Ar. The reaction mixture was allowed to reflux for 4-5 hours in an oil bath and the reaction was monitored using TLC (7:2 DCM:EtOAc) till there was no starting material, which was after about 4 hours. The reaction was stopped by removing the flask from the oil bath and left to cool to room temperature overnight. After a few days, the solution was evaporated to dryness using the rotary evaporator leaving a yellowish white solid. A sample was taken for MS analysis and the rest was continued further to the next step due to instability of the acyl chloride product. Table 5 shows the % yields of step 2 trials performed.

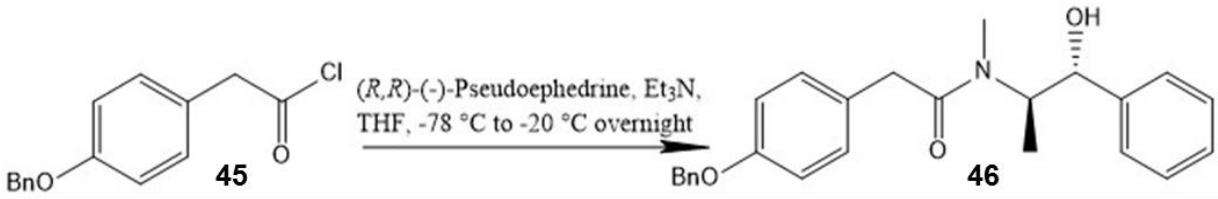
Table 5: %Yields of step 2 trials

Trial	% Yield
1 [249RH43]	90.5
2 [249RH45]	95.6
3 [249RH55]	89.7
4 [249RH83]	99.8

45 [249RH83]:

Yield after drying: 99%; yellowish white solid; EI-MS *m/z* 256, 197, 149, 106, 91; IR 3032 (aromatic CH str.), 2908 (aliphatic CH str.), 1795 (acid chloride C=O str.), 1514, 1246.56, 1012.

3.5 Step 3 [249RH85R]



Scheme 3: Step 3 (attachment of pseudoephedrine chiral auxiliary)

3.5.1 Materials

(*R,R*)-(-)-Pseudoephedrine was purchased from Aldrich. Et_3N was purchased from Merck, which was added to CaH_2, left to dry overnight and distilled in Ar atmosphere immediately before use. CaH_2 was purchased from Fluka.

3.5.2 Method

(*R,R*)-(-)-Pseudoephedrine (3.50g, 1eq.) was weighed into a dry 250ml 2-neck round-bottom flask with a magnetic stirrer and septum. The flask was closed off with an Ar balloon and the air was evacuated using an oil pump followed by flushing with Ar three times. Through the septum, dry THF (70ml) and dry distilled Et_3N (3.5ml) were added. The flask was cooled to -78 ºC by putting it in dry ice/MeOH mixture. In another round-bottom flask, the acyl chloride product from the previous step [249RH83] **45** (5.4g, 1eq.) was dissolved in dry THF (35ml) and cooled in an ice bath. The acyl chloride solution was transferred to the reaction flask through the septum slowly over 45 min. while still keeping it at -78 ºC. The flask was then transferred to the freezer room and kept stirring over weekend. After a few days, the reaction was quenched by pouring the mixture into saturated NH_4Cl (30ml). The product was extracted with DCM (3x30ml). In the first extraction step, the organic DCM layer was the upper layer, while in the second and third steps, it was the lower layer. The organic layers were combined and dried with Na_2SO_4 for 30 min, which was then filtered under vacuum and the filtrate was evaporated to dryness using the rotary evaporator, after which left to dry overnight.

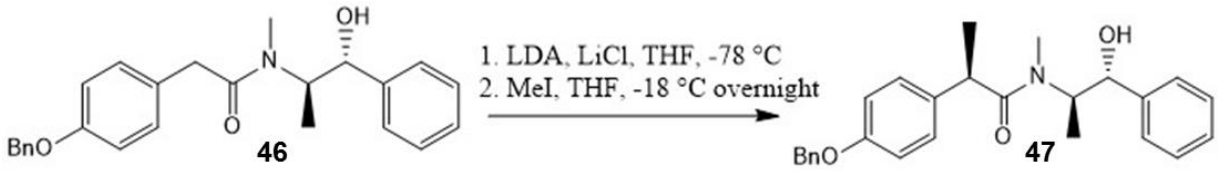
Flash chromatography (2:8 hexane:EtOAc) was used to purify the product. A 4cm-diameter column and 13cm long silica column were enough to separate the product, which eluted first.

After combining the eluted fractions and evaporating the solvent in the rotary evaporator, the product was recrystallized from toluene. A 10 mg/ml solution of **46** in MeOH was prepared to measure its specific optical rotation.

46 [249RH85R]:

Yield: 95% crude, 70% after recrystallization; light white crystals; melting point: 122-127 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.951 (s, 1H), 7.161-7.449 (m, 10 H), 7.093 (d, 2H, *J* = 8.1 Hz), 6.922 (d, 2H, *J* = 7.8 Hz), 5.053 (s, 2H), 4.615 (d, 1H, *J* = 7.8 Hz), 4.435 (m, 1H), 3.626 (s, 2H), 2.806 (s, 3H), 1.133 (d, 3H, *J* = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.720, 157.819, 142.480, 137.192, 129.990 (2C), 128.718 (2C), 128.511 (2C), 128.088 (2C), 127.749, 127.607 (2C), 126.995, 126.531, 115.229 (2C), 58.882, 41.084, 33.535, 27.207, 15.271, 14.503; IR 3100-3400 (small broad OH str.), 1608 (amide C=O str.), 1511, 1243, 1043; $[\alpha]_D^{25}$ = -4.716º.

3.6 Step 4 [249RH87R]



Scheme 4: Step 4 (asymmetric α-methylation)

3.6.1 Materials

n-BuLi and MeI were purchased from Sigma-Aldrich. LiCl and *n*-DIPA were purchased from Merck. 4-Biphenylmethanol 98% was purchased from Aldrich.

3.6.2 Method

First, *n*-BuLi was titrated in order to determine its actual molar concentration. 4-Biphenylmethanol was used as a titrating reagent. At least 80mg was weighed into a 10ml 2-neck round-bottom flask with a magnetic stirrer and rubber septum. The flask was closed off with an Ar balloon and using an oil pump, air was evacuated and flask was flushed with Ar three times. The reagent was dissolved by adding dry THF (5ml) through the septum and

titration was done using 1ml syringe filled with *n*-BuLi. The concentration was calculated using this equation:

$$conc. = \frac{mg\ of\ reagent}{MW\ of\ reagent * ml\ BuLi}$$

The titration was repeated three times and the average concentration was calculated, which turned out to be 1.41 M, from which the required volume was calculated.

LDA (2.1eq.) was then prepared in-situ from *n*-DIPA and *n*-BuLi. A 50ml 2-neck round-bottom flask with a magnetic stirrer, rubber septum and Ar balloon was connected to an oil pump. Air was evacuated and flask was flushed with Ar three times. Through the septum, *n*-DIPA (2.5ml, 2.25eq.) and dry THF (20ml) were added. The mixture was cooled to -78 ºC by placing it in dry ice/MeOH. *n*-BuLi (11.5ml, 2.1eq.) was carefully added through the septum and the mixture was left to warm up to 0 ºC in an ice bath while continuing stirring for 30 min.

For the asymmetric methylation, LiCl (2.00g, 6eq.) was weighed into a dry 250ml 2-neck round-bottom flask with a magnetic stirrer and rubber septum. The flask was closed off with Ar balloon and connected to an oil pump to evacuate air and flush the flask with Ar three times. Through the septum, dry THF (60ml) and the LDA previously prepared were added into the reaction flask under Ar. The mixture was cooled to -78 ºC by placing it in dry ice/MeOH. In a separate 100ml dry round-bottom flask, the pseudoephedrine amide product **46** from previous step [249RH85R] (3.00g, 1eq.) was weighed, dissolved in dry THF (10ml) in Ar atmosphere and cooled to 0 ºC. The amide solution was then added to the reaction mixture gradually over 30 min. at -78 ºC. Stirring was continued at -78 ºC for 1 hr., then for a further 15 min. at 0 ºC. MeI (2ml, 4eq.) was added through the septum all at once and the mixture was left stirring in ice/water bath (0-4 ºC) overnight.

The next day, the reaction was monitored by TLC (2:8 hexane:EtOAc), then quenched by pouring the mixture into 10% Na_2CO_3 (30ml). The product was extracted with DCM (3 x 25ml). The organic layers were combined and dried with Na_2SO_4 for 30 min, which was then filtered under vacuum and the filtrate was evaporated to dryness using the rotary evaporator, after which left to dry over weekend. Flash chromatography (2:8 hexane:EtOAc) was used to purify the product. The eluted fractions were combined and solvent was evaporated to dryness

with the rotary evaporator leaving a light yellow oil, which was further dried with an oil pump. A 10 mg/ml solution of **47** in MeOH was prepared to measure its specific optical rotation. Table 6 shows the % yields of step 4 trials performed along with adjustments in reagents.

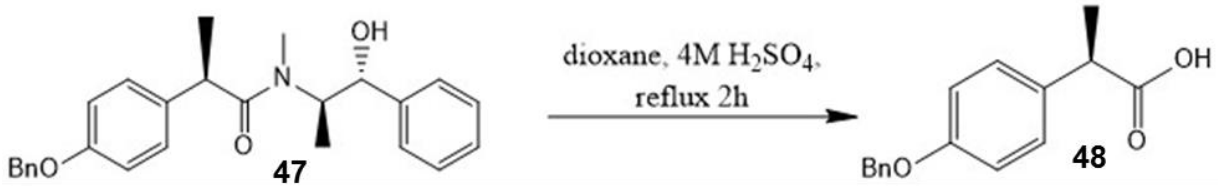
Table 6: %Yields of step 4 trials

Trial	% Yield	MeI (eq.)	LDA (eq.)
1 [249RH61]	39.7	0.4	0.2
2 [249RH87R]	70	4	2.1

47 [249RH87R]:

Yield: 105% crude, 70% after flash chromatography; light yellow sticky solid; melting point: 98-108 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.311-7.418 (m, 10 H), 7.161 (d, 2H, *J* = 8.7 Hz), 6.927 (d, 2H, *J* = 8.7 Hz), 5.043 (s, 2H), 4.575 (q, 1H, *J* = 7.8 Hz), 4.51 (d, 1H, broad), 4.448 (m, 1H), 2.688 (s, 3H), 1.396 (d, 3H, *J* = 6.9 Hz), 1.120 (d, 3H, *J* = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.405, 157.907, 142.53, 137.193, 133.958, 128.937 (2C), 128.767 (2C), 128.533 (2C), 128.157, 127.684 (2C), 127.003, 126.563 (2C), 115.446 (2C), 59.305, 43.578, 33.221, 27.498, 20.992, 14.726, 14.304; EI-MS *m/z* 296, 238, 211, 148, 106, 91, 77; $[\alpha]_D^{25}$ = -2.032º.

3.7 Step 5 [249RH93R]



Scheme 5: Step 5 (hydrolysis of pseudoephedrine chiral auxiliary)

3.7.1 Materials

Dioxane was purchased from Lab Scan Analytical Sciences. Concentrated H_2SO_4 was purchased from J. T. Baker. KOH was purchased from Merck.

3.7.2 Method

Methylated amide from the previous step **47** [249Rh87R] (0.90g) was weighed into a dry round-bottom flask and dissolved in dioxane (20ml). The solution was then added slowly to cooled 4M H_2SO_4 (20ml) in a dry 100ml 2-neck round-bottom flask with a rubber septum. A reflux condenser, which was previously flushed with Ar, with a $CaCl_2$ tube was connected to the 2-neck reaction flask and the reaction mixture was left to reflux in an oil bath for a few hours. The reaction was monitored with TLC (2:8 hexane:EtOAc), which showed the reaction to be complete after 2.5hrs and the solution turned dark red brown in color. The flask was taken out of the oil bath and left to cool overnight. The next day, the mixture was poured into distilled water (30ml) and pH was adjusted to pH 12 with aq. KOH. The mixture was washed with EtOAc (3 x 20ml) to extract the released pseudoephedrine. The aqueous layer was then adjusted to pH below 3 with 4M H_2SO_4 and the product was extracted with DCM (5 x 20ml). The organic layers were combined and dried with Na_2SO_4 for 30 min, which was then filtered under vacuum and the filtrate was evaporated to dryness using the rotary evaporator, after which left to dry overnight.

Flash chromatography (7:2 DCM:EtOAc) was first used to purify the product. The product did not come out of the column, and so switched the mobile phase to 7:2:2 DCM:EtOAc:acetic acid. The eluted fractions were combined and solvent was evaporated to dryness with the

rotary evaporator leaving a yellow oil, which was further dried with an oil pump. Table 7 shows the % yields of step 5 trials performed.

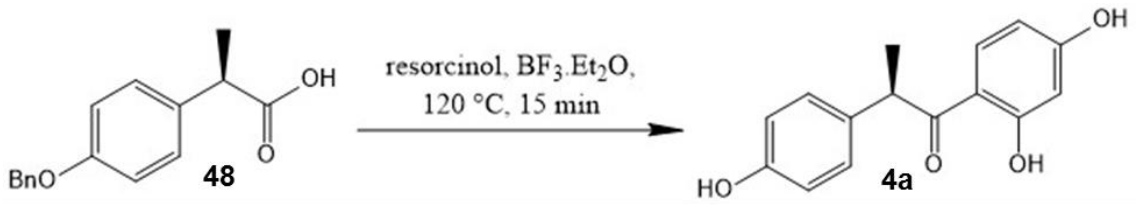
Table 7: %Yields of step 5 trials

Trial	% Yield	Notes
1 [249RH67]	54	No flash chromatography was done
2 [249RH93R]	26.7	

48 [249RH93R]:

Yield: 64% crude, 27% after flash chromatography; yellow oil; ^{1}H NMR (300 MHz, acetone-*d6*) δ 7.256 (2H), 7.164 (2H), 7.089 (1H), 7.023 (dd, 2H, *J* = 2.4 Hz, 8.6 Hz), 6.787 (d, 2H, *J* = 8.6 Hz), 3.959 (s, 2H), 3.645 (q, 1H, *J* = 6.6 Hz), 1.39 (d, 3H, *J* = 6.6 Hz); ^{13}C NMR (75 MHz, acetone-*d6*) δ 176.35, 157.56, 133.29, 130.92 (2C), 129.91 (2C), 129.58 (2C), 129.25, 127.28, 126.76, 116.36 (2C), 45.25, 19.49.

3.8 Step 6 [249RH95R]



Scheme 6: Step 6 (O-DMA synthesis by Friedel-Crafts acylation)

3.8.1 Materials

Resorcinol and $BF_3.Et_2O$ (purified by redistillation, ≥46.5% BF3 basis) were purchased from Sigma-Aldrich. $NaHCO_3$ was purchased from Merck.

3.8.2 Method

Resorcinol (0.134g, 2.02eq.) was weighed into a dry 25ml 2-neck round-bottom flask with a magnetic stirrer and rubber septum. A reflux condenser with an Ar balloon was connected and closed off the flask. Using an oil pump, air was evacuated and Ar was introduced three times. In another dry round-bottom flask, the R-acid from the previous step [249RH93R] **42** (154mg, 1eq.) was dissolved in minimum amount of Et_2O and added through the septum into the reaction flask under Ar. Then, $BF_3.Et_2O$ (0.23ml, 3.05eq.) was added through the septum and the reaction was allowed to reflux in an oil bath at 120 ºC for 15 min. the flask was taken out of the oil bath and left to cool to room temperature. 10% $NaHCO_3$ (10ml) was added to quench the reaction followed by extraction with Et_2O (3 x 10ml). The organic layers were combined, washed with distilled water (10ml), then brine (10ml) and dried with Na_2SO_4 for 30 min, which was then filtered under vacuum and the filtrate was evaporated to dryness using the rotary evaporator, after which left to dry overnight. TLC (7:2 DCM:EtOAc) was done to make sure the reaction was complete and check the solvent system to be used for flash chromatography to purify the product. A 2.5cm diameter column and 20cm layer silica were used. The eluted fractions were combined and solvent was evaporated to dryness with the rotary evaporator leaving a brown oil, which was further dried with an oil pump. After leaving it to dry overnight, a brown solid was formed. A 10 mg/ml solution of **4a** in MeOH was

prepared to measure its specific optical rotation. Table 8 shows the % yields of step 6 trials performed.

Table 8: %Yields of step 6 trials

Trial	% Yield	Notes
1 [249RH75]	13	Reaction was done at 70ºC overnight
2 [249RH95R]	55	Reaction was done at 120ºC for 15 min.

4a [249H95R]:

Yield: 183% crude, 55% after flash chromatography; brown solid; melting point: 127-133 ºC (lit.[23]: 139-140 ºC); ^{1}H NMR (300 MHz, acetone-*d6*) δ 12.889 (s, 1H), 9.383 (s, 1H), 8.229 (s, 1H), 7.908 (d, 1H, *J* = 9 Hz), 7.207 (d, 2H, *J* = 8.4 Hz), 6.785 (d, 2H, *J* = 8.7 Hz), 6.360 (dd, 1H, *J* = 8.7Hz, 2.4Hz) 6.300 (d, 1H, *J* = 2.4Hz), 4.778 (q, 1H, *J* = 6.8 Hz), 1.437 (d, 3H, *J* = 6.9 Hz); ^{13}C NMR (75 MHz, acetone-*d6*) δ 205.500, 166.315, 164.704, 156.556, 133.386, 133.082, 128.797 (2C), 115.837 (2C), 112.427, 107.987, 103.019, 45.556, 18.878; EI-MS *m/z* 258 ($[M+H]^+$), 137, 121, 91, 77; $[\alpha]_D^{25}$ = -6.112º (lit.[7]: -135.8 º).

4 Results and discussion

4.1 General

The main purpose of this work was to develop a stereoselective synthetic route of enantiopure (*R*)-(-)-*O*-DMA. As explained previously, the chirality is present at C-2, thus the suggestion of starting from the precursor 4-hydroxyphenylacetic acid **43** (Figure 29), which would undergo asymmetric methylation at the *α*-carbon to give the (*R*)-2-(4-hydroxyphenyl)propionic acid followed by Friedel Craft acylation with resorcinol. Alkylation can be achieved using enolate chemistry taking advantage of the neighboring carbonyl functional group, while using a chiral auxiliary to obtain the desired stereochemistry. Since pseudoephedrine amides proved to be excellent substrates for these types of alkylation reactions as well as their ease of hydrolysis, (*R,R*)-(-)-Pseudoephedrine was chosen as a suitable chiral auxiliary. Scheme 7 depicts the full reaction series of this multistep synthesis.

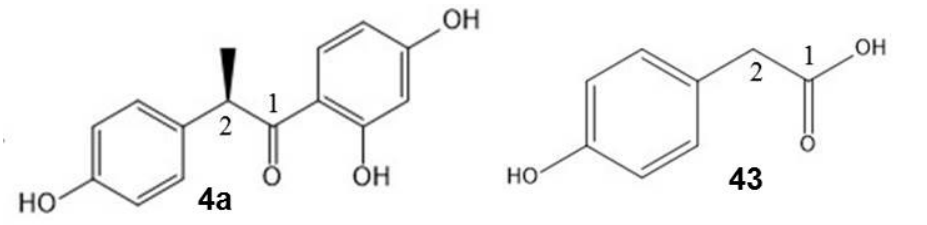
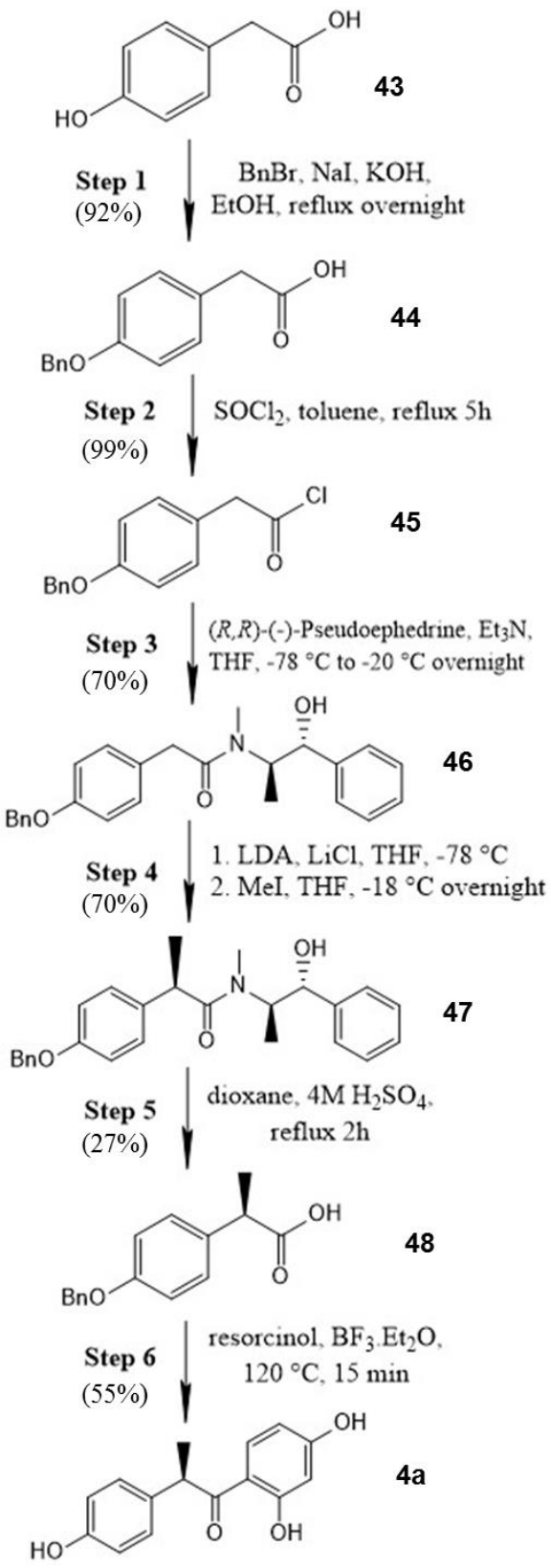
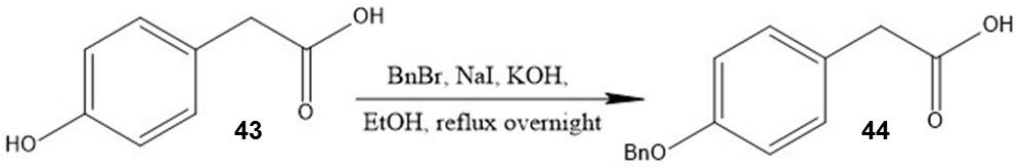


Figure 29: Structures of (R)-(-)-O-DMA and 4-hydroxyphenylacetic acid



Scheme 7: Synthetic route outline of (R)-(-)-O-DMA

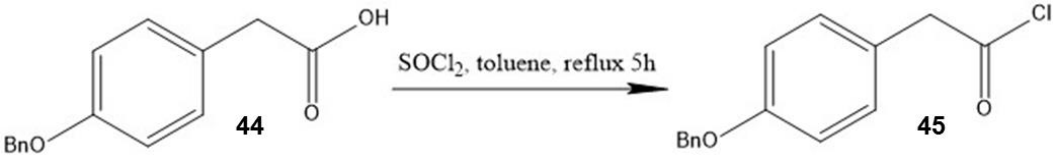
4.2 Step 1



Scheme 1: Step 1 (benzyl protection)

To avoid unwanted side reactions or products, firstly the aromatic hydroxyl group should be protected. Benzyl protection was employed using benzyl bromide in basic conditions. The reaction was straightforward giving a pure white powder with a yield of over 90%. EI-MS showed the expected molecular ion peak at *m/z* 242 (See Appendix 3). ^{1}H and ^{13}C NMR spectra also confirmed the presence of the desired product **38** (See Appendix 1 and 2).

4.3 Step 2



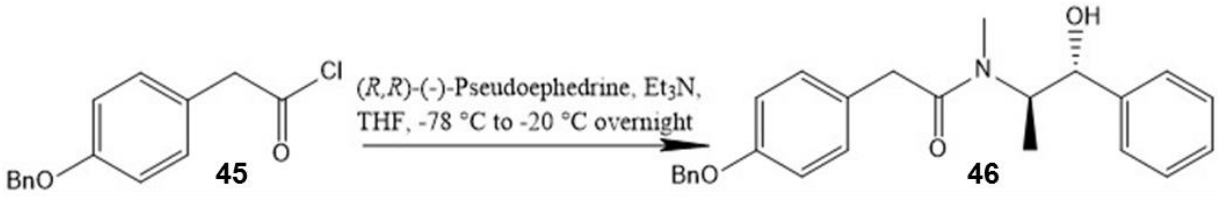
Scheme 2: Step 2 (acid chloride formation)

In order to attach the pseudoephedrine chiral auxiliary, the carboxylic acid group should initially be activated. In this case, it was converted to acid chloride by refluxing with thionyl chloride in toluene. An important precaution in this reaction is that all equipment and solvents have to be completely dry, thus thionyl chloride and toluene were distilled immediately before use and kept under inert Ar atmosphere. In earlier attempts, the solution color turned dark red during reflux and TLC showed many impurities. It was assumed the reason was the presence of some moisture, therefore when repeating extra caution was held to avoid any contact with air and moisture during distillation and setting up of the reaction. TLC showed much better purity and the color was yellow to orange.

The acid chloride product is extremely moisture and air sensitive, so the next reaction should proceed afterwards directly. If not possible, the reaction mixture was left in solution and

evaporated to dryness only before carrying on further. Also, it is not feasible to prepare samples for NMR analysis due to product instability. However, a small sample was taken for IR and EI-MS analysis to make sure the reaction was successful (See Appendix 5 and 6). IR spectrum showed no sign of the broad peak characteristic for carboxylic acid OH group and only showed the sharp peak caused by the carbonyl group at 1800 cm^{-1} (See Appendix 6).

4.4 Step 3

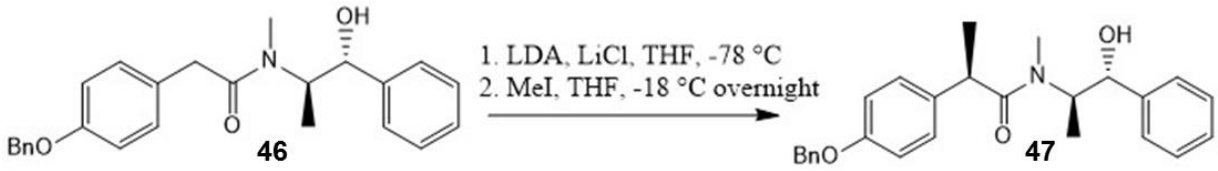


Scheme 3: Step 3 (attachment of pseudoephedrine chiral auxiliary)

After benzyl protection of phenolic hydroxyl group and activation of the carboxylic acid group into an acid chloride, the pseudoephedrine chiral auxiliary can be attached. According to literature, stereoselective α-alkylation of pseudoephedrine amides occurs on the same side as the pseudoephedrine methyl group[24]; and so the (*R*,*R*)-(-) enantiomer was selected to finally yield the pure (*R*)-*O*-DMA isomer. Formation of the amide was done using triethylamine as base in THF as solvent. Similar to the previous reaction, moisture should be eliminated; hence triethylamine was left on CaH_2 overnight, distilled immediately before use and covered with anhydrous $CaCl_2$ tube. Also, THF was dispensed from a solvent purifier machine just prior use and kept under inert Ar atmosphere to ensure the absence of any moisture.

As expected, the acylation reaction occurred readily with a small amount of impurity that is easily removed by flash chromatography. The product is easily recrystallized from toluene and a relatively high yield of 70% of white fluffy crystals was obtained after flash chromatography and recrystallization. The crystals are moisture and air-stable, thus could be conveniently stored in a closed container in the fridge for long-term use.

4.5 Step 4



Scheme 4: Step 4 (asymmetric α-methylation)

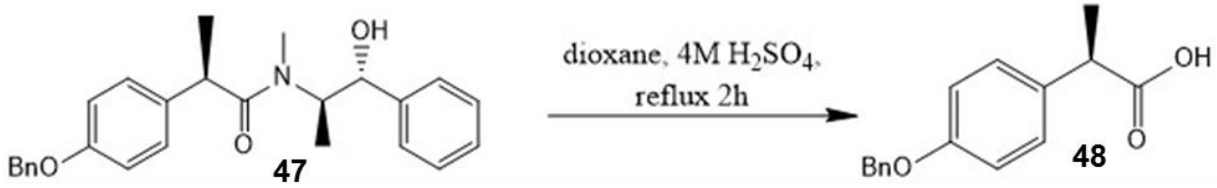
Afterwards comes the most important and main objective of this work, which is the asymmetric methylation of the *α*-carbon. The pseudoephedrine amide enolate was formed by the reverse addition of the amide to an excess amount of base, LDA, which was prepared *in-situ*, followed by addition of excess alkylating agent, which is methyl iodide in this case. LiCl was added to the LDA suspension to accelerate the reaction as stated in previous studies.[24]

Initially, the reaction failed; where the TLC revealed only the starting material along with many impurities. Also, ^{1}H NMR spectrum did not show the expected peak for the presumably added methyl group. After further research, it was believed that the presence of some moisture in THF could be the explanation. When repeating, extra care was taken by keeping all solvents and chemicals under inert Ar atmosphere at all times, especially when dispensing the THF from the solvent purifier. To keep the THF dry, the collecting flask was closed off with an Ar balloon and the required amount was withdrawn through the septum with a syringe. Only then, the reaction was successful; TLC showed an additional spot and an extra doublet peak at around 1.4 ppm appeared in the ^{1}H NMR spectrum as expected (See Appendix 11). However, there were still a lot of impurities and unreacted amide, which decreased the yield to less than 40% after flash chromatography.

In an effort to increase the yield and purity, the amounts of LDA, LiCl and MeI used were adjusted to 2.1 eq., 6 equiv. and 4 equiv. respectively (refer to Table 6). The reaction was repeated with larger amount of starting material (3g instead of 1g), while the procedure and all other aspects remained unchanged. TLC showed much less impurities and a larger product yield; and indeed after flash chromatography, the yield was found to be 70%.

All trials of the reaction yielded an oily product, which when completely dry became a sticky solid/semisolid. This made any attempt to recrystallize the product very hard to achieve. Although the reaction was a success, nevertheless the broad melting point range and unwanted peaks in the NMR spectra indicate residual impurities. Perhaps, this could be the reason for not getting a solid product and failure to recrystallize. Also, as per literatre[55], the quality of *n*-BuLi affects the enantioselectivity and possibly the yield as well, therefore this could also be another reason for the presence of impurities and the low enantioselectivity of the added methyl group.

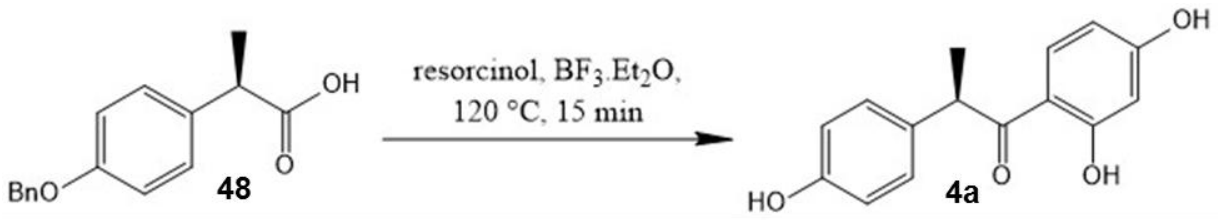
4.6 Step 5



Scheme 5: Step 5 (hydrolysis of pseudoephedrine chiral auxiliary)

Hydrolysis of the amide and release of the pseudoephedrine to give enantiopure (*R*)-acid **42** was done by acidic hydrolysis in 1:1 mixture of dioxane:H_2SO_4. It was noticed that the color always turns dark orange/red during reflux. Evaporating the solvent to dryness leaves off dark red oil, which contains many impurities shown in the TLC and ^{1}H NMR spectrum. Recrystallization failed and was not sufficient to remove the impurities. Flash chromatography was necessary to purify the product enough to proceed with the next reaction. However, this caused a significant loss of the product (yield: 27%), yet still left with yellow oil, lighter in color. It might possibly be that this method of hydrolysis could be too harsh causing some side reactions giving unwanted byproducts and decreasing the yield. Other milder procedures, such as: basic hydrolysis or using Lewis acidic metal salts, should be studied to find an alternative approach to release the pseudoephedrine with higher purity and yield. Though, it should be kept in mind the product stability, cost and reaction time.

4.7 Step 6



Scheme 6: Step 6 (O-DMA synthesis by Friedel-Crafts acylation)

The final reaction in this multistep synthesis can be proceeded, which is Friedel-Crafts acylation of **42** with resorcinol in $BF_3.Et_2O$, similar to racemic *O*-DMA synthesis as explained in literature.[21-23] The same protocol and conditions were employed; however the final yield after flash chromatography was merely 55% (literature recorded at least 80%[21-23]). This could be due to the presence of residual impurities from previous steps. It should also be noted that purification by flash chromatography was rather difficult; the impurities eluted very close to the product and some loss was inevitable in order to get pure *O*-DMA. This was observed in racemic *O*-DMA synthesis as well, though a considerably higher yield was obtained (91%), which is comparable to previous references.

EI-MS showed the expected molecular ion peak at *m/z* 258 alongside with typical *O*-DMA fragment ion peaks (See Appendix 18).[8] Also, both ^{1}H and ^{13}C NMR spectra exhibited results identical to racemic *O*-DMA synthesized by Friedel-Crafts acylation as well as those found in literature (See Appendix 16 and 17).[8] The measured $[\alpha]_D^{25}$ was found to have a negative value (-6.112º) as expected, which proves the (*R*)-(-) optical nature of the synthesized *O*-DMA. However, by comparing to the value published in literature ($[\alpha]_D^{25}$ = -135.8º)[7], %ee was calculated to be 4.5%. The equation $\%ee = \frac{experimental\ [\alpha]_D^{25}}{reported\ [\alpha]_D^{25}} * 100$ was used to calculate the %ee. Attempts to recrystallize the product from 1:1 Et_2O:*n*-hexane were not successful; rate of recrystallization was too rapid and pale yellow amorphous crystals were formed, which are not suitable for X-ray crystallography. Similar trials on racemic *O*-DMA also failed. Alternative solvents/solvent mixtures for recrystallization should be further studied and larger amounts of product should be available for better and more reproducible results.

5 Conclusion

O-Desmethylangolensin has been and still is studied extensively with respect to its biological activities and potential health benefits. Since recently, it has been identified that (*R*)-(-)-*O*-DMA is the major enantiomeric form produced by intestinal bacteria[7], hence it would be useful to develop an organic synthetic route to synthesize this *O*-DMA enantiomer in a chiral fashion, which was the main purpose of this work. (*R*,*R*)-(-)-Pseudoephedrine was used as a chiral auxiliary for subsequent diastereoselective *α*-methylation. The proposed method was found to be successful; (*R*)-(-)-*O*-DMA was eventually produced, yet the %ee was very low.

As mentioned in literature, the pseudoephedrine amide was easily formed yielding in pure stable white crystals. However, there were several difficulties concerning the *α*-methylation step; most importantly the success of the reaction depended on the complete absence of moisture and avoiding its introduction. Moreover, recrystallization of the final product should be investigated further in order to perform X-ray crystallography experiments, which would give a clearer and more precise idea about the stereochemistry and enantioselectivity.

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